Exploring the role of sugar-mediated protein glycation in prostate cancer biology

M.Sc. thesis presented by

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Abstract

A growing body of evidence points to changes in the physical properties of the extracellular matrix (ECM), which forms the backbone of the tissue, as an important driver for most cancers. Notably, stiffer tissues are associated with increased risk of developing tumours over a person's lifespan and more aggressive cancers. Matrices stiffening normally occurs through biological enzymes-mediated crosslinking of ECM components (*e.g.* collagen). However, non-enzymatic reactions such as the Maillard reaction can also crosslink the ECM as well as soluble proteins to generate advanced glycation endproducts (AGEs). This reaction is accelerated by the increase availability of reducing sugars in circulation, which is the main characterizing feature of diabetes. Interestingly, and unlike other cancers, diabetes is associated with increased risk of prostate cancer mortality but is inversely associated with prostate cancer incidence. In our study, we aim to identify a molecular basis to this conundrum by characterizing the interplay between both soluble and immobilized glycated proteins and matrix stiffness in modulating prostate cancer proliferative and invasive capacity.

Our results demonstrate that soluble AGEs decrease cell proliferation in a dose dependent manner across four different prostate cancer cell lines. Interestingly, we show that soluble AGEs do not cause apoptosis and have minimal impact on the cell cycle, which suggests that the observed effect is most likely cytostatic. We also show that the AGEs effect on cell proliferation is long lasting and irreversible, suggestive of cellular senescence or extended phases in the cell cycle. Critically, soluble AGEs treatment decreased the stiffness-mediated induction of cell spreading. Similarly, we show that soluble AGEs also decrease cell migration in a dose-response manner. Finally, bioinformatics analysis of publicly available datasets revealed that a proportion of AGE receptors (AGERs) is differentially expressed in prostate tumours and metastases, adding potential clinical relevance to the observed phenotypes. Altogether, our findings exposed opposing roles for the glycation of soluble proteins and the extracellular matrix stiffness and suggest that the tumour suppressing properties of glycated soluble proteins could be harnessed as a novel therapeutic avenue through precision nutrition.

Résumé

De plus en plus d'études démontrent que les changements physiques dans la matrice extracellulaire (MEC), qui constitue le « squelette » des tissus, sont un facteur important dans l'apparition de la plupart des cancers. Notamment, des tissus plus rigides sont associés à un risque élevé de développement de tumeurs au cours de la vie, ainsi qu'à des cancers plus agressifs. Le raidissement des matrices survient normalement par la réticulation enzymatique des composantes de la MEC (par exemple le collagène). Cependant, les composantes de la MEC peuvent aussi être réticulées par des voies non enzymatiques, comme la réaction de Maillard, qui aboutit aussi à la génération des produits finaux de glycation avancée (AGE). Cette réaction est accélérée par la disponibilité accrue de sucres réducteurs circulant, laquelle est la principale manifestation du diabète. Curieusement, et contrairement à d'autres cancers, le diabète est associé à une mortalité accrue du cancer de la prostate, mais est inversement associé à l'incidence du cancer de la prostate.

Dans cette étude, nous voulons identifier une base moléculaire qui puisse expliquer ce paradoxe, en caractérisant le lien entre les protéines glyquées solubles et immobilisées et la rigidité de la MEC et le potentiel prolifératif et invasif du cancer de la prostate. Nos résultats montrent que les AGEs diminuent la prolifération cellulaire selon une relation dose-dépendante dans 4 lignées cellulaires de cancer de la prostate. Surprenamment, nous montrons que les AGEs solubles ne causent pas l'apoptose et ont un impact minime sur le cycle cellulaire, ce qui suggère que les effets observés sont probablement cytostatiques. Nous démontrons aussi que l'effet des AGEs sur la prolifération est irréversible, ce qui suggère la sénescence ou une extension des phases du cycle cellulaire. En outre, un traitement aux AGEs soluble a diminué l'étalement cellulaire médié par la rigidité de la matrice. Nous montrons, de façon similaire, que les AGEs solubles diminuent la migration cellulaire suivant une relation dose-réponse. Enfin, l'analyse bio-informatique de données publiques a révélé qu'une proportion des récepteurs aux AGEs (AGERs) est différentiellement exprimée dans les tumeurs de la prostate, rendant les phénotypes observés potentiellement pertinents en clinique. En conclusion, nos résultats montrent des rôles opposés de la glycation des protéines solubles versus la rigidification de la matrice, suggérant que les propriétés antitumorales des protéines glyqués solubles peuvent être potentiellement utilisées comme nouvelle avenue thérapeutique dans le contexte de l'intervention nutritionnelle.

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ADT	androgen deprivation therapy
AGE	advanced glycation endproduct
AGER	advanced glycation endproduct receptor
AJCC	american joint committee on cancer
AMPK	monophosphate-activated protein kinase
AR	androgen receptor
ATCC	american type culture collection
BCR	biochemical recurrence
BMI	body mass index
BPE	bovine pituitary extract
BPH	benign prostate hyperplasia
BSA	bovine serum albumin
CDK	chronic kidney disease
CRPC	castration resistant prostate cancer
CSC	cancer stem cell
CSPC	castration sensitive prostate cancer
CYP17A1	cytochrome P450 17A1
DMEM	dulbecco's modified eagle medium
DRE	digital rectal exam
EBRT	external beam radiation therapy
ECM	extracellular matrix
EGF	epidermal growth factor
ELISA	enzyme-linked immunosorbent assay
EMT	epithelial to mesenchymal transition
FBS	fetal bovine serum
FDA	food and drug administration
FGF	fibroblast growth factor
FTIR	Fourier-transform infrared spectroscopy
GEMM	genetically engineered mouse model
GFP	green fluorescent protein
HMGB1	high mobility group box 1
HAS	human serum albumin
HUVEC	human umbilical vein endothelial cell
IGF1	insulin growth factor 1
IL	Interleukin
LDL	low-density lipoproteins
MEC	matrice extracellulaire
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging

NFRF	new frontiers in research fund
PARP	poly-ADP ribose polymerase
PCA	principal component analysis
PCR	polymerase chain reaction
PFA	Paraformaldehyde
PI	propidium iodide
PI3K	phophoinositide 3-kinase
PIN	prostate intraepithelial neoplasia
PSA	prostate specific antigen
PTEN	phophatase and tensin homologue gene
qPCR	quantitative polymerase chain reaction
RAGE	receptor for advanced glycation endproducts
RPMI	Roswell Park Memorial Institute Medium
SAM	S-adenosylmethionine
SNP	single nucleotide polymorphism
TCGA	the cancer genome atlas
TERT	telomerase reverse transcriptase
TMA	tissue microarray
TME	tumour microenvironment
TRAMP	transgenic adenocarcinoma of the mouse prostate

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CONTRIBUTION OF AUTHORS

This thesis follows a manuscript-based structure. I wrote the first, third and fourth chapters under the direction of David P. Labbé.

The presented work in the second chapter is the result of the collaboration between the Labbé laboratory (Tarek Hallal, M.Sc. candidate, Nadia Boufaied, research associate, and David P. Labbé, principal investigator) and the Bordeleau laboratory (Carole Luthold, postdoctoral research fellow and François Bordeleau, principal investigator). This manuscript was primarily written by me and proofed by David P. Labbé. Co-authors wrote the methodology for the sections that they contributed to. Migration and spreading assays were designed, optimized, and performed by Carole Luthold, with the help of François Bordeleau. Proliferation, apoptosis, cell cycle and senescence assays were designed, optimized, and performed by me, with the help of David P. Labbé and Nadia Boufaied. Bioinformatic analysis was conducted by Nadia Boufaied, the datasets were scrutinized and reviewed by me, and selected with the help of Nadia Boufaied and David P. Labbé. Western Blotting, quantitative polymerase chain reaction (qPCR), and spectral analysis were conducted and analyzed by me, and the Fourier-transform infrared spectroscopy (FTIR) was performed and analyzed by Mahmoud Omar.

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Chapter 1: Research background

1.1 Prostate cancer

1.1.1 Prostate Cancer: General Introduction

Prostate cancer is the most diagnosed cancer in Canadian males, and the third leading cause of cancer-related death, after lung and colorectal cancer. The Canadian Cancer Society projects that 23,3000 men will be diagnosed with prostate cancer in 2020, and 4,200 will die from their disease [1]. A plethora of options is available for disease screening, diagnosis, and treatment. However, an overarching challenge in the field remains the lack of concise stratification between indolent and aggressive disease, which leads to overtreatment of the former and poor management of the latter, resulting in therapy resistance and eventually patient death. It is therefore of utmost importance to characterize mechanisms involved in the progression of prostate cancer towards aggressive forms, which could offer physicians better tools to diagnose the disease and to treat it.

1.1.2 Prostate Cancer: disease etiology and drivers

Although the exact origin of human prostate cancer remains elusive, multiple studies hint at a multifactorial etiology for the disease, made even more complicated due to the high heterogeneity observed in prostate tumours [2]. Cellular players such as the cell of origin and prostate cancer stem cells (CSCs), molecular features such genetic aberrations, and environmental cues such as inflammation all provide viable seeds and fertile soil for cancer initiation and progression.

On a cellular level, the prostate gland is composed of three distinct types of epithelial cells. The luminal cells compose most of the epithelial population and secrete several proteins such as the prostate specific antigen (PSA). These cells express the androgen receptor (AR) and are thus responsive to androgens. The basal cells represent a population that lines the basement membrane and is considered less differentiated than its luminal counterpart. Although their exact function is uncertain, it is thought that these cells mostly play a regulatory role as they constitute a barrier within the prostate gland, preventing substances coming from the blood from reaching luminal cells [3]. The third population is a very rare

subtype of cells exhibiting “neuroendocrine” features and expressing distinct markers such as synaptophysin and chromogranin A. Their function remains obscure, and they are thought to minimally contribute to disease initiation. The cell of origin for prostate cancer is a topic that has been hotly debated and heavily investigated throughout the years, often with conflicting conclusions regarding the luminal versus basal origins of the disease. Perhaps the most compelling evidence for a luminal origin of the disease is the fact that prostate adenocarcinomas exhibit luminal phenotypes [4]. One of the methods to address the cell of origin question is by using a lineage-tracing strategy that allows discrimination of luminal vs. basal populations in different genetically engineered mouse models (GEMMs) harbouring genetic features that are represented in human disease and see which population gives rise to tumours in those animals. Wang *et al.* have used this strategy to investigate the cell of origin in three different GEMMS: the *NKx3.1*$^{+/-}$; *Pten*$^{+/-}$ model (also referred to as the NP model) [5], the Hi-MYC model which thrives on a human *MYC* overexpression system [6] and the *TRAMP* model that overexpresses the tumour promoting SV40 large T antigen under a prostate-specific promoter [7]. They used an inducible Cre system to ensure temporal control over the lineage marking and what they observed was that luminal cells are preferred as the cell of origin for prostate cancer under different genetic contexts [8]. On the other hand, evidence for a basal origin of cancer also exists. For instance, Goldstein *et al.* saw that cells of basal origin that are derived from a benign human prostate epithelium are capable of tumour initiation in mice, hinting at a basal origin of the disease in a human context [9]. Undeniably, the cell of origin question is not easy to answer and the different findings in that regard might simply reflect the inherent limitations that are used when trying to isolate different populations and grafting them into mice. The current evidence suggests that both scenarios are plausible, and they might not be mutually exclusive.

Stem cells are defined as a rare population of cells that exhibit distinct functional properties characterized by self-renewal and pluripotency, which is the ability to differentiate into any type of somatic cell. Extremely important during embryogenic development and crucial for tissue regeneration, stem cells assume a less compelling function in the context of cancer, as they are often linked to disease progression and relapse after treatment and referred to as CSCs. The first evidence of a stem-like population in cancer comes from hematological cancers, more specifically acute myeloid leukemia [10]. As for the prostate, Collins *et al.*

were able to isolate such cells from a subset of prostate tumours with varying grades and sites of invasion by enriching cells that express specific stemness markers such as CD44, α2β1, and CD133. They go on to show that these cells represent roughly 0.1% of the entire tumour population and that their number is not correlated with the cancer's grade [11]. Moreover, prostate CSC were shown to harbour tumour-initiating capacities *in vivo* that can be sensitized to chemotherapy by targeting angiogenin and plexin-B2 [12]. The implications of these cells on the disease course and outcomes are immense. In addition to contributing to cancer metastasis and disease recurrence, prostate CSC have also been shown to be more resistant to classical treatments, especially radiotherapy and chemotherapy [13, 14]. This means that despite a robust initial response to treatment, and because the stem cell population is too small to be picked up during routine screening, a lot of patients will go into remission thinking that they have been "cured" before ultimately relapsing and presenting with a more aggressive form of the disease that has already acquired resistance and is thus harder to successfully manage.

On a molecular level, cancer has long been considered a genetic disease, with cells accumulating mutations and insults over time eventually escaping clearance mechanisms and initiating life-threatening cancers, and prostate cancer is no different. Although prostate tumours have relatively fewer somatic point mutations (0.4 per Mb) than most other cancers, such as breast (1.2 per Mb), bladder (7.1 per Mb) and melanoma (12.9 per Mb) [15], the genetic landscape of prostate cancer is mostly shaped by chromosomal rearrangements and copy number alterations leading to the amplification and thus activation of oncogenes as well as the deletion of tumour-suppressor genes. For instance, *MYC*, an oncogene that is often altered in several cancers, has been reported to be activated in up to 50% of prostate tumours, through increase in gene copy number [16] which leads to the upregulation of factors involved in cell growth and proliferation. On the other side, the Phophatase and Tensin homologue gene or *PTEN*, which is a negative regulator of the pro-proliferative phophoinositide 3-kinase (PI3K) pathway, acting as a tumour-suppressor, is partially or completely lost in prostate cancer, mainly through heterozygous and homozygous deletions and less commonly through inactivating mutations [17]. Deletion of the prostate-specific transcription encoding gene *NKX3.1* has also been reported [18] and is considered a tumour-initiating event according to GEMMs, where *NKX3.1* deletion leads to formation of prostate

intraepithelial neoplasia (PIN) lesions [19], an event widely accepted as a precursor to human prostate cancer [20] . Both somatic and germline alterations in DNA repair genes such as *BRCA1*, *BRCA2* and *ATM* have been observed in patients with advanced disease [21]. With sequencing getting more high-throughput and cheaper, cancer genomics will continue to help identify key genetic features that will revolutionize the field of personalized medicine, by using the genomic landscape of each cancer patient to gain more insight regarding his prognosis, his likelihood of developing aggressive disease, and to guide treatments that are more targeted and thus more effective.

In 2011, Hanahan and Weinberg published a follow-up review to their famous and highly cited seminal work entitled "The hallmarks of cancer" where they propose two "emerging hallmarks" as well as two "enabling characteristics" [22]. One of these characteristics is tumour-enabling inflammation, as immune and endothelial cells within the tumour-microenvironment act as mediators that facilitate growth, angiogenesis, and invasion by sending pro-proliferative signals and secreting ECM remodeling enzymes and pro-angiogenic factors. While an anti-tumour immune response does exist, inflammation within tumour sites is paradoxically favourable for growth and metastasis. Moreover, chronic inflammation has been shown to be associated with the onset of certain cancers such as colorectal cancer. As for the prostate, a prospective study that examined over 65,000 men from different ethnic backgrounds found a positive association between a history of prostatitis (the inflammation of the prostate) and prostate cancer risk [23]. A proposed mechanistic explanation for this observation is termed "cytokine addiction" where an influx of interleukin-6 (IL-6) in the tumour micromilieu can trigger prostate CSCs to drive disease initiation and progression [2].

1.1.3 Prostate cancer risk factors

Despite the current lack of a definite etiological clue to prostate cancer incidence, several epidemiological studies have outlined factors that increase a man's risk of contracting prostate cancer, the most established being age, ethnicity, family history and genetic factors. However, diet and lifestyle factors are being widely accepted as modifiable risk factors for prostate cancer [24].

Age:

Prostate cancer is undeniably a disease of old age, with the average age of diagnosis being at 67 years old, and the risk significantly increasing after the age of 50 [25]. In fact, almost 60% of cases are diagnosed at 65 or later, and less than 1% of cases are below the age of 45 [26] which underlines the role of age in disease incidence. Intriguingly, autopsy studies have found microscopic prostate lesions in men in their forties [27]; the role of those lesions in disease evolution and appearance later in life is yet to be determined. In older men, incidental autopsy-detected prostate cancer is far more common, with a prevalence nearing 50% in men over the age of 90 [28]. However, these tumours are generally considered clinically insignificant, due to their low mean volume, their confinement to the organ, and their unlikelihood to metastasize. Nevertheless, the study of these tumours might prove useful, providing valuable insight into disease etiology, and why certain men die with prostate cancer while others die because of it.

Ethnicity:

Ethnicity is considered an important risk factor for prostate cancer, with Asian men having the lowest incidence and mortality rates and African Americans being the most affected. For example, in the United States, men of Asian ancestry have a risk of incidence of 88.3 per 100,000 of the population which increase up to 2.5 fold in African American men [25], with mortality rates following similar trends [26]. In fact, men of African ancestry also show higher serum PSA levels upon diagnosis [29] and greater Gleason scores of tumours detected during autopsies [30]. A plethora of reasons could account for the racial disparity in prostate cancer, ranging from molecular features such as differences in prostate cancer single nucleotide polymorphism (SNP) frequency [31] to socioeconomic factors such as neighbourhood disadvantage [32] and medical mistrust [33].

Family history:

Epidemiological studies have long considered family history an established risk factor for prostate cancer. In fact, men can expect their risk to increase between 2 and 3 fold when having a first-degree family member (such as a father or a brother) diagnosed with the disease [24], with the risk positively correlating with the total number of family members

affected and negatively with the age of disease onset [25]. Interestingly, a meta-analysis conducted in 2019 found that first-degree family history of breast cancer could increase prostate cancer risk and lethality [34], hinting at probable common genetic factors governing the etiology of both diseases.

Genetic factors:

Prostate cancer can be classified as one of the three: either 1) sporadic, with no familial pattern whatsoever, 2) familial, when two or more first-degree relatives are affected or 3) hereditary, a specific subset of familial prostate cancer where cases follow a Mendelian inheritance pattern [35]. Therefore, genetic factors appear to play an important role in disease appearance. In fact, genome wide analyses have uncovered several prostate cancer-related genes, with the vast majority showing a dominant autosomal inheritance pattern [36]. These genes are mostly involved in DNA repair mechanisms and mutations in *BRCA1*, *BRCA2*, *HOXB13*, *CHEK2*, *PALB2* and *NBS1* are often found [37]. Additionally, SNPs found in several genomic regions are associated with prostate cancer, and the risk increases with the cumulative number of SNPs found, regardless of family history [38].

Apart from inherited gene mutations, epigenetic studies are pointing at DNA methylation and histone modifications in disease initiation, progression, and recurrence. For instance, a study published in 2008 showed that CpG islands hypermethylation in more than five gene loci positively correlates with biochemical recurrence after radical prostatectomy [39]. Moreover, in prostate cancer, several alterations in the patterns of histone modification are observed, with respect to acetylation, methylation, and phosphorylation [40].

Diet and lifestyle factors:

An epidemiological migration study of Japanese Immigrants has found that their prostate cancer incidence rate is almost identical to that of US-born residents, which is up to 4-fold of the homeland population's rate, suggesting a role for environmental factors in prostate cancer etiology and progression [41]. In fact, diet seems to play a role in prostate cancer progression, with some foods offering protection while others fuelling the disease towards aggressivity and increasing mortality. For instance, epidemiological studies have found that obesity, which is defined by an elevated body mass index (BMI), is associated with an increased biochemical recurrence (BCR) and prostate cancer lethality [42]. Additionally,

high saturated fat intake was linked to an increased all-cause death risk among non-metastatic prostate cancer patients [43], and the molecular underpinning is just beginning to uncover. Using a MYC-driven murine prostate cancer model, Labbé *et al.* show that high saturated fat intake leads to an increase in disease progression and metabolome alteration even in precancerous PIN lesions, setting the stage for an epigenetic reprogramming that impacts both cellular proliferation and tumour burden [44], which suggests that the effects of the diet could be long-lasting, and acting early on during disease onset. Indeed, diet is thought to affect prostate cancer epigenome by providing metabolites that act as substrates or cofactors for epigenetic regulatory enzymes: for example, S-adenosylmethionine (SAM) which is derived from the essential amino acid methionine, acts as a methyl donor for methyltransferases, a group of enzymes responsible for DNA and histone methylation: a hallmark of epigenetic regulation [45].

On the other hand, several studies have found that the consumption of certain food components could significantly decrease the risk of prostate cancer. For instance, consumption of tomatoes and tomato-based products can have a protective effect that seems to be stronger for aggressive disease [46], the underlying mechanism thought to be due to lycopene, a strong antioxidant that was already shown to inhibit prostate cancer cell proliferation *in vitro*, as well as normal prostate cells [47, 48]. Moreover, a broccoli-rich diet was shown to prevent or delay adenocarcinomas in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model, which was associated with a decrease in histone deacetylase 3 (HDAC3) protein expression in the prostate epithelium at 12 weeks of age, suggesting a diet-related early epigenetic underpinning of the disease initiation and progression [49].

Altogether, evidence cumulated from both preclinical and human studies shows that diet is an important factor that can strongly mediate disease etiology and progression, with the mechanistic explanations only beginning to be elucidated. Diet being the only modifiable risk factor for prostate cancer makes it of utmost importance as it could potentially be leveraged to prevent disease, delay its course, or even sensitize tumours to treatment.

1.1.4 Prostate cancer: screening and diagnosis

Several screening methods have been adopted to detect prostate cancer early to offer better control of the disease. PSA is a protein secreted by epithelial cells of prostate origin. In 1991, Catalona *et al.* showed that, when used in combination with other means of disease detection such as digital rectal examination (DRE) and ultrasonography, serum PSA measurement is a useful tool to detect prostate cancer [50]. Three years later, the Food and Drug Administration (FDA) approved the use of PSA testing in men over 50, when used in combination with DRE. However, it is important to bear in mind that PSA can be elevated in other non-cancer conditions, such as benign prostatic hyperplasia (BPH) and prostate inflammation or prostatitis, meaning that PSA results should not be treated as the sole decision-making tool to diagnose prostate cancer. When screening tests give abnormal results (*i.e.,* elevated serum PSA and/or an abnormal DRE), physicians often require additional tests to confirm a prostate cancer diagnosis. These tests include a rectal ultrasound or Magnetic Resonance Imaging (MRI) to create a clear image of the gland, in addition to prostate biopsies which examines the tissue for the presence of cancerous lesions.

1.1.5 Prostate cancer staging and grading

Upon prostate cancer diagnosis, tests are conducted to see how contained the disease is, whether other parts of the body are affected, and to determine the likelihood of the cancer to spread (if it has not already). To stage prostate cancer, the TNM system, developed by the American Joint Committee on Cancer (AJCC), is used. It looks at three main features of the disease: the first being the size of the tumour (T) and whether it is still constrained within the gland, the second involves any lymph node (N) invasion, and the third looks at possible sites of metastasis (M). There are five stages for the disease, ranging from zero (the least advanced) which is highly curable to four (the most advanced) which is considered the deadliest.

Another method to classify prostate cancer is the Gleason grading system, which has undergone several modifications over the years and is still commonly used among physicians to determine the aggressiveness of the disease. It consists of looking at tumour tissue under the microscope and comparing the appearance of the cancer cells to that of healthy cells to characterize how differentiated they are: cells that look similar to healthy cells (*i.e.*, well

differentiated) are attributed low scores and those that look very different (*i.e.*, poorly differentiated) are given high scores. Scores from two distinct regions: the first called the primary pattern, which is the dominant pattern of the tumour, and the second called the secondary pattern, which is the second most observed pattern in the biopsy, are added to come up with a number ranging from six to ten, with six representing tumours that are slow-growing aTnd unlikely to spread (Gleason grade group 1) while ten describes tumours that show signs of aggressiveness and are likely to metastasize (Gleason grade group 5) [51].

1.1.6 Prostate cancer treatments

Prostate cancer treatment modalities depend on disease stage and aggressiveness, as well as patient's age and general health condition. They include:

- Active surveillance: also called watchful waiting, this option's main goal is to delay treatment and maintain the quality of life of patients who have localized and slow-growing disease for as long as possible, all while monitoring them with the intent to intervene should any changes in the disease course arise. This approach has been shown to be both safe and effective, as many cohort studies demonstrated very low prostate cancer risk mortality and metastasis risks when this method was employed [52, 53].
- Radical prostatectomy: it is the surgical removal of the prostate gland along with surrounding tissue (*i.e.*, seminal vesicles and lymph nodes) with the intent to cure localized disease. According to two population-based cohort studies, surgery is generally associated with some long-term effects such as decrease in sexual function and urinary control compared to men on active surveillance [54, 55].
- External beam radiation therapy (EBRT): this treatment involves using ionizing radiation pointed at the prostate to kill cancer cells. It is curative for localized disease and palliative for more advanced forms. Newer techniques are becoming more precise which means healthy tissue is spared and fewer side effects are observed [56].
- Brachytherapy: also called internal radiation, this procedure involved placing a temporary radioactive source (high-dose-rate brachytherapy) or permanent seeds (low-dose-rate brachytherapy) inside the patient's prostate to deliver radiation in a focalized manner [57]. There are two types of brachytherapy: low-dose and high-dose. This treatment is

generally used for early-stage indolent prostate cancer. Side effects include urinary problems and bowel dysfunction, that may resolve over time.

- Androgen deprivation therapy (ADT): In 1941, Charles Huggins revolutionized the field of metastatic prostate cancer treatment when he showed that androgen ablation, achieved via bilateral orchiectomy, is associated with a decrease in serum phosphatases, which are usually elevated when patients have bone metastases. On the other hand, he showed that androgen injection can have the opposite effect [58], demonstrating for the first time that cancer can be systemically controlled by hormonal manipulation, which awarded him the Nobel Prize for Physiology or Medicine in 1966. To this day, androgen deprivation therapy continues to be used as a first line treatment for metastatic prostate cancer. It is achieved either by surgical castration (*i.e.*, the removal of the testes or orchiectomy), or more frequently by chemical means, by using drugs that suppress or decrease androgen production in the body. Most commonly used drugs include gonadotropin-releasing hormone (GnRH) agonists and antagonists that block androgen secretion within the hypothalamus-pituitary-gonadal axis, where up to 95% of androgens is produced, the remaining being produced at the level of adrenal glands [59]. This treatment is not without risks, with a lot of patients on ADT experiencing mild symptoms such as fatigue and mood swings as well as more serious complications like metabolic changes [60] and osteoporosis (loss of bone density) [61]. Although initially very successful at shrinking tumours and managing disease, some patients on ADT will eventually relapse and develop what is known as castration resistant prostate cancer or CRPC. At this stage, the disease has often spread outside the prostate and is referred to as metastatic castration resistant prostate cancer (mCRPC). However, it is important to note that some metastatic cancers are hormone sensitive, which is the case of cancers that are stage IV at the time of diagnosis: they are referred to as metastatic castration sensitive prostate cancers (mCSPC).
- Second-generation AR-targeted therapies: Since prostate cancer can be fueled by extragonadal androgens, which are spared by classical ADT, complete depletion of androgen biosynthesis is necessary to halt the disease progression. Abiraterone acetate, a drug that blocks androgen biosynthesis by inhibiting the activity of the cytochrome P450

17A1 (CYP17A1) enzyme [62], was approved by the FDA in 2011 and is used today to treat mCRPC. Enzalutamide, an AR inhibitor, is approved for both mCRPC and mCSPC.

- Chemotherapy: When patients develop resistance to androgen and AR-targeted therapies, chemotherapy is the preferred treatment modality. Taxanes are a class of anticancer drugs that impair microtubule dynamics, inhibiting cell mitosis and causing cancer cells to die by apoptosis. Docetaxel, a taxane, is generally used when hormone treatments fail. However, most patients become docetaxel resistant during or after treatment, and are then treated with carbazitaxel [63]. As for patients with mCSPC, the use of ADT alone has been considered the standard of treatment however, several studies have reported positive effects on overall survival for combining ADT with docetaxel or second-generation hormone therapy drugs (such as abiraterone and enzalutamide) [64, 65]. The use of chemotherapy for localized disease is currently limited.
- Therapies under development: the prostate cancer treatment scene is constantly changing, with new therapies being tested and developed, and well-established treatments being tuned and perfected. Perhaps one of the most exciting emerging cancer treatments is immunotherapy, which harnesses the body's own defence mechanisms to fight tumour cells However, this road has proven to be bumpy, especially for prostate cancer, which is considered a "cold tumour" with a low T-cell infiltration, making it particularly resistant to immune directed therapies. In fact, there are currently only two FDA-approved prostate cancer immunotherapies. Sipuleucel-T is a cancer vaccine that activates anti-tumour cellular response by using a prostate antigen fused to an immune activator and showed improved survival in mCRPC patients [66]. Pembrolizumab, an immune checkpoint inhibitor, is used after all other treatment options are exhausted. Another interesting new avenue in prostate cancer treatment is the use of poly-ADP ribose polymerase (PARP) inhibitors, which take advantage of tumour cells synthetic lethality in a DNA damage context. As of May 2020, the FDA approved two PARP inhibitor drugs for use in men with prostate cancer harbouring certain DNA repair gene mutations that showed efficacy in clinical trials: olaparib and rucaparib [67]. In addition, the use of radioactive isotopes has emerged as a treatment option for men with metastatic disease. Prostate specific membrane antigen (PMSA) targeted radioligands appear to be both safe and effective, as a cohort study showed a notable decrease (around 50%) in PSA levels

among men with mCRPC, following a single dose of ^{177}Lu-PMSA-617 treatment [68]. In June 2021, the FDA granted ^{177}Lu-PMSA-617 Breakthrough Therapy Designation, which is given to novel drugs or therapies that treat life-threatening conditions and are shown to provide clinical benefit over currently approved treatments.

1.1.7 Current prostate cancer care limitations

Despite the latency of prostate cancer disease when compared to other tumours, it is still a leading cause of mortality in Canadian men [1]. One of the most important limitations in prostate cancer care is the lack of well-established tools that offer accurate distinction between indolent and aggressive tumours, which hinders accurate prognostic determination and complicate treatment decisions, negatively impacting oncological outcomes and patient's overall survival [69]. Another important challenge in the prostate cancer treatment scene is the acquired resistance, where treatments that were once successful in managing the disease would stop working for the patient, who inevitably progresses to a metastatic, and often fatal stage. Therefore, there is a pressing need for new biomarkers that allow better disease stratification which would result in better disease management, as well as the need for new treatments to address the resistance of the current ones, until we hopefully learn how to outsmart cancer.

1.1.8 Diabetes and cancer: a special case for the prostate

In the 1920s, almost 30 years before the DNA double helix was described and characterized by Watson and Crick, German physiologist Otto Warburg first noted that cancer cells have an altered metabolism characterized with high glucose consumption and increased lactate excretion, even in the presence of oxygen [70], which would be known as the "Warburg effect" and considered an "emerging" hallmark of most if not all cancers [22]. Bearing this mind, it would make perfect sense for diabetes, the most prevalent metabolic disease worldwide, to affect cancer initiation, progression, prognosis, and treatment. Indeed, clinical studies undeniably show a clear relationship between cancer and diabetes: a pooled analysis of almost 100 prospective cohort studies concluded that diabetic patients can see their risk of cancer mortality increase up to 25%, with liver and pancreatic cancers showing the highest magnitude, while ovarian and colon cancer showed only a moderate association, and lung and breast cancer showing the lowest [71]. Several mechanisms have been proposed to

explain the positive association between the two diseases, one being related to insulin, which has been shown to exert pro-mitotic properties on epithelial cells by acting on its own receptor and subsequently activating the mammalian target of rapamycin (mTOR) pathway [72]. Another plausible explanation is related to pro-inflammatory cytokines (such as interleukin-1 or IL-1, IL-6 and interleukin-15 or IL-15) that are secreted by clustered adipocytes and help bridge inflammation and cancer [73]. In addition, adiponectin, the most abundant adipokine in the plasma, harbours antioxidant and anti-tumour properties and is found to be reduced in obese and diabetic patients. [71]

When it comes to diabetes and prostate cancer, the data is controversial. Unlike other types of cancer, meta-analyses have reported that type 2 diabetes is associated with a statistically significant decreased risk of developing prostate cancer [74]. Although the underlying biological causes remain for the most part elusive, many suggested that this effect might be due to the low circulating androgen levels in diabetic patients, which can provide a protective effect against prostate cancer [71]. Another plausible explanation would be the effects of anti-diabetic drugs which can also yield better oncological outcomes. For example, metformin, a first line treatment for diabetes that has been employed for decades, is known to have a protective effect against prostate cancer, mainly through adenosine monophosphate-activated protein kinase (AMPK) dependent mechanisms, as well as effects that are linked to insulin growth factor 1 (IGF1) suppression and mTOR inhibition [75, 76]. However, despite this rather encouraging data regarding the protective role of diabetes and its treatment against prostate cancer, a meta-analysis has found that diabetes can increase mortality rate in diabetic men diagnosed with prostate cancer up to 30% [77]. Importantly, during the early stages of disease development, prostate cancer cells do not exhibit a “Warburg phenotype” as they rely primarily on lipid metabolism for energy uptake, and it is only during the late metastatic stages of disease progression that cancer cells switch and start requiring increased glucose for their energetic requirements [78]. To conclude, more studies are needed to explain and confirm the disparity between prostate cancer and other types of cancer with respect to diabetes, accounting for both types as well as treatment modalities, which would help develop better strategies for disease treatment and management.

1.2 Glycation

1.2.1 Introduction to glycation: The Maillard reaction

In 1912, French physician and chemist Louis-Camille Maillard first described a slow non-enzymatic reaction that happens between carbonyl groups of reducing sugars (e.g., glucose, fructose, etc.) and the amino group of proteins, which would later be known as the "Maillard Reaction". Initially, this reaction leads to the formation of an unstable product, the "Schiff's base" which can undergo atomic rearrangement in order to generate a more stable and advanced class of molecules, referred to as the "Amadori products", which can also undergo rearrangement, among other reactions such as dehydration and cyclization, leading ultimately to the formation of advanced glycation endproducts (AGEs) (**Figure 1-1)** [79]. Hyperglycemia, oxidative stress, aging, as well as renal disease are all known to trigger the formation of endogenous AGEs in the human body [80]. In fact, glycation can affect a great range of molecules, from proteins to DNA to lipids. For example, the glycated form of hemoglobin (Hb1AC) was the first example of non-enzymatic glycation *in vivo*, first reported in the 1960s in diabetic patients, is now used to monitor the long-term glycemic control in those patients and to assess their risk for cardiovascular disease [81]. It is the relatively long half-life of the Hb1AC hemoglobin molecule, estimated at 25-35 days [82], that makes long-term glycemic monitoring (2-3 months) possible. Another physiologically relevant target of glycation is the long-living proteins of the ECM, such as collagen and elastin, since their slow biological turnover can make them highly prone to the Maillard reaction and AGEs accumulation. Once glycated, these proteins form glycation crosslinks which mechanically alter the architecture of the matrix, leading to increased tissue stiffness and loss of elasticity [83, 84].

Interestingly, glycation has been proposed as a theory to explain aging. In aging people, which are often also diabetic, increasing concentration of reducing sugars leads to an increase in glycation rate and subsequently sugar-modified proteins which have been linked to several age and diabetes-related pathologies such as cataracts, atherosclerosis, and neuropathy [85]. Moreover, sugars can also interact with DNA causing mutagenesis and damage. In fact, DNA-AGEs have been shown to cause mutagenesis in human fibroblasts, which can be partly reversed through the nuclear excision repair (NER) pathway [86]. Therefore, defects in this pathway can render cells particularly sensitive to the mutagenic effect of DNA-AGEs,

which in turn fuels genomic instability, a key enabling characteristic for cancers [22]. Thus, glycation might provide a mechanistic explanation for the increase likelihood of cancer occurrence with aging.

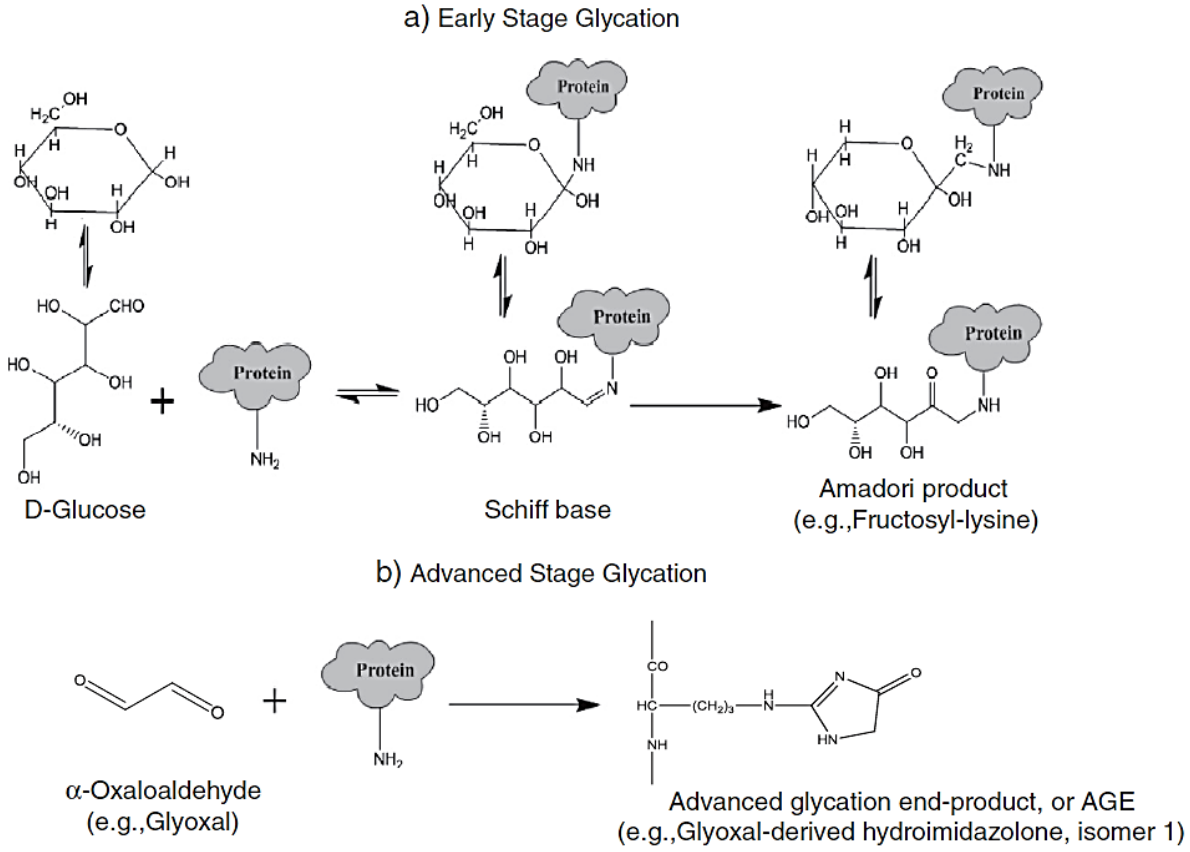

Figure 1-1: The glycation reaction is a multi-step process. It starts with the formation of a Schiff base, followed by an Amadori product, leading ultimately to the formation of AGEs [87]. Used with permission from Elsevier with Rightslink® (License number : 5121451347274).

1.2.2 Glycation as a clinical biomarker: methods to measure albumin glycation

Due to their suspected effects on different pathologies, the study of glycated proteins represented a captivating area of research. While certain angles have been thoroughly investigated, such as the study of the glycation of long-living proteins (collagen) and hemoglobin, there is a growing interest in studying other targets of glycation, such as human serum albumin (HSA), the most abundant plasma protein, and the closely related bovine serum albumin (BSA). HSA has a molecular weight of 67 kDa and assumes many biological functions, including but not limited to osmotic regulation in blood, lipid metabolism, drug binding, as well as antioxidant properties. Structurally, this serum protein has a globular

shape and is mostly composed of α-helices [87]. It is rich in lysine residues and to a lesser extent in arginine residues, which can both act as potential sites of glycation. Due to its high abundance in the blood and its relatively high half-life (estimated at 18 days), HSA is a target of glycation and is found to be elevated in its glycated form among diabetic patients, with a rate as high as 90% in patients with no glycemic control [88]. Therefore, glycated serum measurement can be used as a clinical biomarker to indirectly monitor long-term hyperglycemia and estimate the rates of glycation *in vivo*. Glycation can also be successfully conducted *in vitro*, and the process is simple and straightforward. It involves incubating serum albumin in the presence of a reducing sugar of choice, which acts as a glycation reagent, under sterile conditions for an extended amount of time (could range from several days to several weeks) at a physiological temperature (37℃). Ribose, glucose, and methylglyoxal are often used to mediate glycation, and the browning or yellowing of the solution is used as a visual indicator of successful glycation. Several methods have been developed to quantify the level of glycation, *in vitro* and *in vivo*. Some of these methods include:

- Fructosamine enzymatic assay: this assay is used to measure the level of fructoasmine (a glycated molecule) in serum samples. It is used in clinical settings and is based on the enzymatic activity of ketoamine oxidase, which leads to the release of hydrogen proxide that can be quantified and used to estimate glycation levels, usually expressed as a percentage of the total serum albumin sample [89]. An initial protein digestion step is required prior to conducting the assay and the calibration is done against reference material.
- AGE antibodies, carboxymethyllysine or CML: CML is a cleavage product that occurs during the Maillard reaction and it is widely accepted in the literature as an AGE marker and epitope. CML antibodies are commercially available and can be used to estimate the level of glycation in protein lysates obtained from different biological specimen (by Western Blot), in soluble specimens (by enzyme-linked immunosorbent assay or ELISA), also in tissues (by immunohistochemistry). For example, CML antibodies have been used to show an increase in AGE accumulation in diet-induced obese and diabetic mice [90].
- Mass spectrometry: Mass spectrometry (MS) is a technique that measures the mass-to-charge ratio of ions and is useful to accurately determine the molecular mass of proteins.

Because glycation only causes subtle changes in protein mass, using MS to assess glycation in full-length albumin molecules is challenging. While MS generally requires an enzymatic digestion step, glycated samples need to be thoroughly broken down to expose the monomers and make the distinction between glycated and non-glycated specimen less challenging [91].

- Raman spectroscopy: Raman spectroscopy is an optical non-destructive vibrational method that allows the characterization of molecular structure and composition. And because glycation is associated with chemical and structural changes in molecules, Raman spectroscopy can be employed to differentiate between glycated and non-glycated entities. Alsamad *et al.* showed that Raman can be used to successfully assess collagen glycation *in vitro* when coupled with additional post-hoc analyses such as principal component analysis (PCA) and Lasso regression [92].
- Fourier-transform infrared spectroscopy (FTIR): this method is similar to Raman spectroscopy with some differences. While Raman measures scattered light when it passes through a sample, FTIR is an absorbance technique that generates easily interpretable spectral profiles that allow discrimination of glycated molecules due to the presence of certain peaks that can be attributed to glycation within the tested sample [93].

1.2.3 Effects of advanced glycation endproducts (AGEs) in disease models: non-cancer

The effect of AGEs has been studied in both cancer and non-cancer disease models. Outside of cancer, AGEs are often used to study diabetes-related conditions and complications as well as neurodegenerative diseases. With the aim to investigate the molecular underpinning of atherosclerosis pathophysiology, Li *et al.* assessed the effect of commercially purchased AGEs on human umbilical vein endothelial cells (HUVEC) behaviour [94]. What they saw was a dose and a time dependent decrease in proliferation as well as a decrease in cell migration. They show that the effect is mediated by lysosomal protein Cathepsin D downregulation. Similarly, another group showed that glycoaldehyde-derived AGEs block mitochondrial function and proliferation capacity of rat fibroblasts [95]. Interestingly, the authors show that the treatment alters the expression of certain ECM-related genes, hinting at a possible crosstalk between glycated proteins and ECM remodeling. Moreover, in a neurodegenerative disease model, ribose-derived AGEs were shown to specifically block proliferation and cause protein misfolding and aggregation of SH-SY5Y neuronal cells,

leading to toxicity-related cell death [96]. Finally, glucose-derived AGEs were found to induce autophagy in rat cardiomyocytes [97], providing a plausible mechanistic explanation for the cardiomyopathy observed in diabetic patients. In summary, studies that looked at the effect of AGEs on cellular behaviour in a non-cancer context generally reached similar conclusions where AGEs seem to have a cytotoxic effect characterized by a decrease in proliferation and migration and an increase in cell death and autophagy.

1.2.4 Effect of advanced glycation endproducts (AGEs) in disease models: cancer

While AGEs seem to negatively impact non-cancer cell viability and migration potential, several studies have demonstrated opposite results in cancer settings, meaning that AGEs assume pro-tumourigenic functions characterized with an increase in proliferation, colony formation and cancer invasion, with evidence coming from breast, liver, prostate, as well as other types of cancer. For instance, in an *in vitro* breast cancer model, methylglyoxal derived BSA AGEs caused an increase in proliferation, invasion and migration of the highly invasive triple negative cell-line MDA-MB-231 [98]. The treatment was also shown to enhance matrix metalloproteinase 9 (MMP-9) activity, which might explain the increase in invasive and migrative potential. However, in a hormone-sensitive model (MCF-7), the same group of authors showed that the AGEs effect depends on the dose that is used for the treatment: under low concentrations (50-100 μg/mL), cell proliferation, measured by cell count, was increased whereas the opposite phenotype was seen when a higher concentration was used (200 μg/mL) [99]. This study showcases how experimental design can completely change biological outcomes which is important to bear in mind especially when the data is conflicting. Moreover, a retrospective cohort study that followed almost 200,000 women over the course of 13 years found a link between dietary AGE intake and the risk of developing invasive post-menopausal breast cancer [100]. However, in this study, dietary AGEs intake was estimated from food-frequency questionnaires, and since food that are highest in AGEs contents are often unhealthy and rich in saturated fat [101], it is difficult to decouple the effect of AGEs from other food components that could be exerting cancer promoting properties. The effect of AGEs in cancer has also been investigated *in vivo.* Glucose AGEs were found to be elevated in colon cancer patients' sera when compared to healthy controls, and to promote liver metastasis in mice [102]. In addition, AGE levels, measured by ELISA, were shown to be elevated in the saliva of multiple myeloma patients

who have bone lesions [103], implying that AGEs can be used not only as a therapeutic target, but also as a biomarker to predict disease aggressiveness. In prostate cancer, commercial AGEs induced PC-3 cell proliferation by stimulating the retinoblastoma protein (Rb) phosphorylation and degradation [104]. Intriguingly, a mouse study found that early glycation endproducts, rather than AGEs, support prostate tumour growth *in vivo* through immune modulation of tumour-associated macrophages [105]. In conclusion, current evidence suggests that AGEs might be implicated in phenotypes associated with tumourigenesis such as proliferation, migration, invasion, and metastasis. However, the results seem to be heavily dependent on the cell lines, the type of AGEs, and the concentrations that are used for the treatments which is illustrated by the intriguing dichotomy between the effects seen in cancer *vs*. non-cancer models.

1.3 The extracellular matrix

1.3.1 The extracellular matrix: in sickness and in health

The extracellular matrix (ECM) is a dense, three-dimensional network of macromolecules that surrounds cells and supports tissues physical integrity, as well as their dynamics and homeostasis. There are two main types of ECM that are present within tissues: the interstitial matrix, which is present within the connective tissue, and the basement membrane, which separates epithelial cells from the underlying stroma. Proteoglycans (PGs) and fibrous proteins (such as collagen, elastin and fibronectin) constitute the building blocks of the matrix, as shown in **Figure 1-2**.

The critical role of ECM in maintaining tissue integrity under normal physiological conditions is accentuated by developmental studies that have shown that genetic deletions of ECM proteins are often embryonic lethal [106]. Healthy ECM homeostasis is executed and maintained through the cooperation of three major players: the substrate, the effectors and the sensors [107]. The substrate, also referred to as the “matrisome” regroups up to 300 proteins that, by interacting with each other, maintain tissue architecture [108]. For example, elastic fibres, which are networks of amorphous elastin and surrounding fibrillin-based microfibrils, are known to provide several tissues with elasticity, extensibility and resilience [109]. With over 25 types, collagen is the most abundant protein in the human body. Unlike the elastic fibres, collagen confer tissues with features of stiffness and strength [107]. Fibroblasts are

considered the main effectors of the ECM architectural organization as they synthesize, secrete and regulate most of its components [110]. Finally, a class of transmembrane heterodimeric proteins, called integrins, link the ECM to the intracellular actin cytoskeleton, exerting functions of mechanosensing and mechanotransduction [106, 107].

The ECM is a dynamic entity, constantly undergoing remodeling and turnover where synthesis of new building blocks and degradation of ECM molecules are tightly regulated. Diseases emerge once this balance is perturbed. For example, cancer and fibrosis are characterized by abnormal increase in ECM deposition while on the other hand, excessive degradation of ECM materials leads to conditions such as osteoarthritis (**Figure 1-2**) [106, 111].

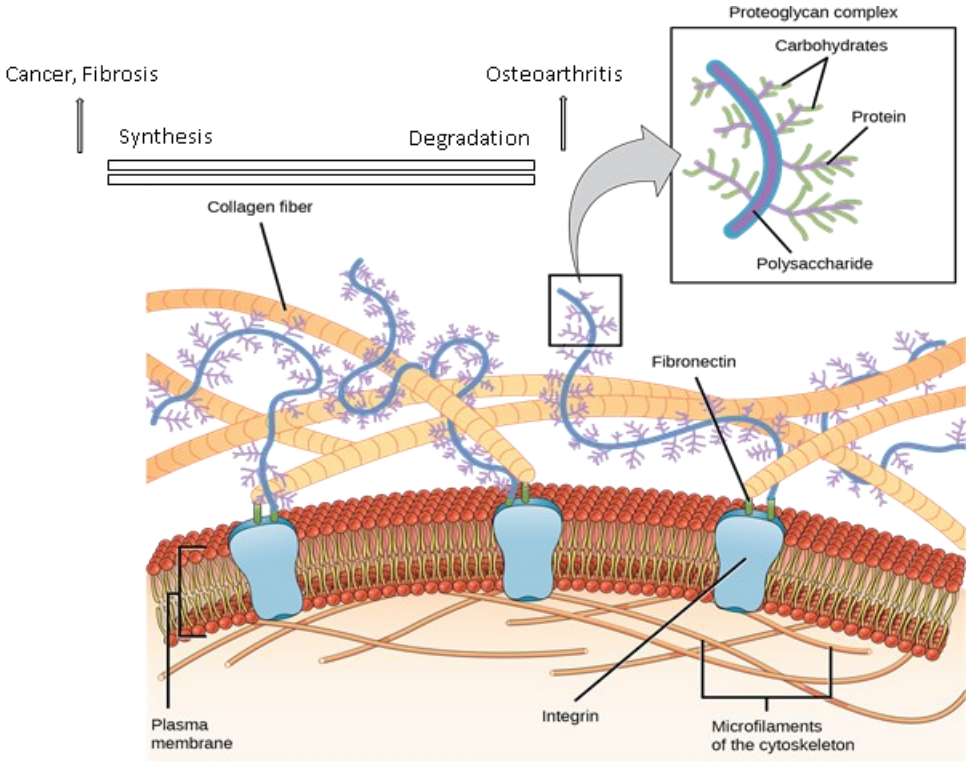

Figure 1-2: The extracellular matrix is a dynamic entity. The ECM is characterized by continuous turnover of different constituents such as collagen, fibrous proteins, and proteoglycans. Adapted from from Biology 2e. Textbook content produced by OpenStax is licensed under a Creative Commons Attribution License 4.0. https://openstax.org/books/biology-2e/pages/4-6-connections-between-cells-and-cellular-activities.

1.3.2 Collagen: key component of the extracellular matrix

Collagens designate a family of fibrous proteins that constitute the building blocks of the ECM: in fact, collagen is the most abundant type of protein in the animal world. It is secreted mostly by fibroblasts, which are connective tissue cells, as well as other cell types such as epithelial cells [112]. Collagen is first synthesized as a precursor molecule, called procollagen, which is then enzymatically cleaved, staggered, then crosslinked into the active and stable form. Structurally, a typical mature collagen molecule contains three polypeptide chains, the α chains, which are assembled in a long rope-like triple helix. There are many types of collagens in the body, however fibrillar collagens (such as collagen I) are by far the most common as they are found in the bone, skin, and internal organs (such as the prostate), conferring both strength and elasticity to the tissue they underly. Being a key component of the ECM, collagen has been largely used in biomaterial development, as it provides natural scaffolding for matrices. Because of its native biological properties, collagen is often used in cell culture to mimic a more physiologically relevant environment, and to promote cell adhesion and proliferation. Sources of used collagen vary from classical sources like animal and even human tissues to more sophisticated means such as cell-produced collagen and completely synthetic collagen [113].

Collagen homeostasis is a tightly regulated mechanism, critical for maintaining tissue health and integrity. When this balance between collagen production and degradation is perturbed, tissue architecture is compromised which gives rise to age-related complications as well as several diseases, including cancer. Crosslinking, which is defined as the formation of covalent chemical bonds between adjacent matrix fibrils, is one example of this perturbation. This process is regulated by an enzyme, Lysyl Oxidase (LOX), but it can also happen by non-enzymatic means. As alluded to in section 2-a, collagen can be a target of the Maillard reaction, becoming glycated and crosslinked. In fact, the slow biological turnover of collagen (cartilage collagen's half-life has been estimated to be at 117 years [114]) makes it the perfect target for the already slow Maillard reaction since proteins with longer half-lives are more susceptible to become glycated and reach the final step of the reaction, which is AGE

formation. Moreover, glycated collagen has been shown to alter cell morphology and to promote live migration in a breast cancer model [115].

1.3.3 Matrix stiffness and cancer

With one of the earliest detectable signs of most solid cancers being a palpable mass, tissue stiffness is at the core of the disease etiology and progression. In fact, tumours are often found to have aberrant matrices which are thought to play major roles in both tumour initiation and progression. For example, a study noted that primary breast carcinomas can be stratified based on ECM profiles, suggesting a link between stroma characteristics and cancer progression and clinical outcomes [116]. A year a later, Levental *et al.* showed that ECM stiffening, caused primarily by collagen crosslinking, promotes breast cancer progression *in vivo* by regulating integrin activity as well as focal adhesion formation and subsequent mediated signaling [117]. In fact, the extracellular matrix is argued to mediate all hallmarks of cancer [118]. An aberrant ECM can sustain proliferation by facilitating cell cycle transition [119, 120], and help tumours evade apoptosis through Bax inactivation and Bcl2 upregulation [121, 122]. In addition, matrix stiffening supports angiogenic sprouting and promotes tumour vasculature formation [123]. It can also drive cancer invasion and metastasis through β-catenin induced epithelial to mesenchymal transition (EMT) [124]. Indeed, matrix stiffening is emerging as a compelling mechanism to explain the origin of most cancers, since the processes that govern matrix alteration are thought to be conserved and consistent, compared to the more cell-centric events that accompany cancer [125].

1.4 Receptors for advanced glycation end products (RAGE)

1.4.1 RAGE: one receptor, multiple ligands

The receptor for advanced glycation end products (RAGE) belongs to the immunoglobulin (Ig) superfamily of transmembrane receptors [126, 127]. It is involved in a myriad of diseases, ranging from diabetes [128] to cancer [129]. Under normal physiological conditions, RAGE expression levels are generally very low in cells and tissues [130] with the exception of lung and aortic smooth muscle cells which show higher mRNA RAGE levels [131]. These levels are known to increase under pathological conditions.

RAGE is a multi-ligand receptor, as it interacts with several molecules, eliciting multiple cell responses and activating different signalling pathways. Those ligands include advanced

glycation end products (AGEs) which were the first identified RAGE ligands and generated through the Maillard reaction, high mobility group box 1 (HMGB1)/amphoterin, S100 group of proteins or calgranulins, as well as damage-associated pattern molecules (DAMPs), among others [130]. RAGE ability to bind a variety of ligand is thought to be due to pattern recognition, as all its ligands share two common features: a net negative charge and the ability to oligomerize [130]. Ligand binding causes the recruitment of intracellular mediators such as Diaphenous-1 (Dia1) [132], which leads to the activation of several subsequent signaling pathways, such as Ras/MAPK, NF-κB and Rac/cdc42 and subsequently affecting a wide array of cellular mechanisms including proliferation, inflammation and migration [133].

1.4.2 RAGE in cancer

RAGE expression was shown to be elevated in various tumours, including breast, colon, liver, prostate, stomach, pancreatic, as well as lymphoma and melanoma [134], which makes targeting RAGE for cancer therapeutics and diagnostics an interesting avenue. Indeed, data from Kwak *et al.* identifies RAGE as a key driver of breast tumour cell invasiveness *in vitro* and *in vivo* [135]. In liver cancer, RAGE was found to promote cancer cell proliferation as well as drug resistance [136]. As for stomach cancer, RAGE expression correlated with more aggressive disease and poor patient outcomes [137]. Additionally, the HMGB1/RAGE axis was found to induce pancreatic tumour growth through mitochondrial bioenergetic modulation [138]. Finally, RAGE was shown to promote the proliferative effect of AGEs on PC-3 prostate cancer cells through the Akt pathway [104]. It appears clearly that RAGE and its ligands are involved in a wide array of cancer types. However, the exact mechanisms by which RAGE, by interacting with its ligands, drives the observed phenotype of cancer progression and metastasis remain elusive and open to speculation. One proposed explanation is inflammation, which was proposed in 2011 as a cancer "enabling characteristic" capable of promoting tumours towards proliferation, invasion and metastasis, by providing the suitable niche of growth factors, metalloproteinases, and antiapoptotic molecules [22]. Interestingly, RAGE is known to be a strong mediator of inflammation, mainly through the upregulation of NF-κB [133]. Following this logic, once activated by one of its ligands, RAGE can promote a positive feedback loop of sustained inflammation that creates a tumour enabling microenvironment that further drives cancer towards a state of invasion and aggressivity. Additionally, NF-κB can induce telomerase through the nuclear

translocation of the telomerase reverse transcriptase (hTERT) [139], which provides tumours with replicative immortality, a “hallmark” of cancer where cells can divide indefinitely [140]. Despite the abundant and compelling data suggesting a role for RAGE and its ligands in cancer progression, there is still a need to identify how specific ligands, including AGEs, drive certain cancer phenotypes, and whether the observed phenotypes can be due to RAGE ligand binding *per se*.

1.4.3 Other AGE receptors

Apart from the most studied AGE receptor (RAGE), other receptors are known to interact and bind AGEs, which are grouped under two major families: the AGE-R complex family and the macrophage scavenger receptor family [141].

The AGE-R complex comprises three AGE-binding proteins: p60, also known as oligosaccharide transferase 48 (OST-48) or simply AGE-R1, protein kinase substrate C 80K-H or p90 (AGE-R2) and galectin-3 or AGE-R3. The first two receptors were initially isolated from rat liver membranes, and later found to be present on the membrane of different cell types such as monocytes, endothelial cells, and neurons [142]. Little is known about their functionality. A study on chronic kidney disease (CDK) patients found that AGE-R1 levels are decreased in patients when compared to healthy individuals, suggesting an antioxidant role under normal circumstances [143]. On the other hand, p90 was found to undergo tyrosine phosphorylation in the context of fibroblast growth factor (FGF) signalling [144]. The third component of the AGE-R complex, galectin-3, belongs to the lectin family of sugar-binding proteins. In 1995, galectin-3 was found to bind AGEs with high-affinity, however, its lack of transmembrane domain suggests that it might be linked to other members of the complex of the cell surface [145]. Interestingly, this protein can exert anti apoptotic functions in different cancers [146, 147], which rendered it an attractive target for cancer therapies.

The scavenger family amasses a broad range of receptors that were first thought to interact with modified low-density lipoproteins (LDL), though they are now known to recognize and bind a variety of ligands, including AGEs. The SR-A receptor (scavenger receptor class A) can modulate the endocytosis and degradation of AGEs, as well as acetyl-LDL [148]. Similar results were reported for CD36 and SR-BI proteins, members of the class B [149, 150].

Another member of this family, the lectin-like oxidized low-density lipoprotein 1 receptor (LOX-1) was identified as an endothelial receptor for AGEs [151]. Additionally, AGEs can upregulate LOX-1 expression in endothelial cells and metformin, a anti hyperglycemic drug, can block this effect [152]. Laminin-type EGF-like, and link domain-containing scavenger receptor-1 (FEEL- 1) and its paralog FEEL-2 were also identified as endocytic receptors for AGEs [153].

Apart from galectin-3 [154] and CD36 [155], little is known about these "other" AGE receptors. Therefore, the roles they might play in cancer are poorly understood and are yet to be fully elucidated.

1.5 Research objectives

1.5.1 Rationale

Despite huge breakthroughs in screening, diagnosis, and treatment, prostate cancer remains one of the most common cancers in North American men and a leading cause of cancer-related mortality. Diabetes, another widespread disease worldwide, could affect cancer course and oncological outcomes, according to an increasing body of epidemiological evidence [156]. Several theories have been proposed to bridge the two diseases. Glycation, whose rates are elevated in diabetic patients, affecting a wide array of soluble circulating serum proteins in addition to the proteins of the ECM, could also impact the course of cancer [157].

1.5.2 Hypothesis

We hypothesize that glycation, through its impact on soluble proteins and extracellular matrix modulation, could affect prostate cancer development in diabetic patients.

1.5.3 Objectives

In our study, we aim to characterize the role of glycated proteins and matrix stiffness in mediating prostate cancer aggressive phenotypes. To achieve this objective, we will be addressing the two following main aims:

1) Assess the impact of immobilized glycated proteins on prostate cancer aggressive phenotypes *in vitro.*
2) Assess the impact of soluble glycated proteins on prostate cancer aggressiveness *in vitro.*

It is important to mention that both aims will be addressed in the context of the extracellular matrix modulation, in order to study the effect of stiffness.

1.5.4 Research design and methods

Our experimental design is based on the use of four prostate cancer cell lines of different origins to better capture the multifaceted aspect of the disease. We use three cell lines of human origin, one for early stages (LAPC4) and two for advanced stages (22Rv1 and PC-3), as well as a murine cell line (MyC-CaP) that overexpresses *MYC* [6, 158], an oncogene known to be activated in many cancers, including the prostate [159]. Glycated proteins, soluble and immobilized, are prepared in-house, following established protocols. Matrix stiffness modulation is achieved by using tunable polyacrylamide gels.

1.5.5 Contributions to the advancement of knowledge

This project addresses the knowledge gap in the literature regarding the role of glycation in prostate cancer cellular biology.

Chapter 2

Soluble advanced glycation endproducts block phenotypes associated with prostate cancer aggressiveness

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2.1 Abstract

Changes in the physical properties of the extracellular matrix constitute an important driver for most cancers. Notably, stiffer tissues are associated with increased risk of developing tumours over a person's lifespan and with more aggressive cancers. Glycation, which is elevated under hyperglycemic conditions, leads to physical changes in the ECM as well as the formation of soluble proteins, called advanced glycation endproducts (AGEs). Our results demonstrate that soluble AGEs decrease cell proliferation in a dose-response manner across four different prostate cancer cell lines. Interestingly, we show that soluble AGEs do not cause apoptosis and have no impact on the cell cycle, suggesting that the observed phenotype is most likely cytostatic. We also show that the AGEs effect on cell proliferation is long lasting and irreversible, suggestive of cellular senescence. Critically, soluble AGEs treatment decreased the stiffness-mediated induction of cell spreading. Similarly, we show that soluble AGEs also decrease cell migration in a dose-response manner. Finally, bioinformatics analysis of publicly available datasets revealed that a majority of AGE receptors (AGERs) are differentially expressed upon prostate cancer development, while a subset is significantly associated with altered time to biochemical recurrence.

2.2 Introduction

Prostate cancer is the most common non-skin cancer in the United States and the second leading cause of cancer-related mortality in men, as it is estimated that over 34,000 American men will succumb to the disease in 2021 [1]. Several factors including age, family history, and lifestyle factors contribute to the etiology of the disease. Diabetes is a highly prevalent and rapidly growing disease, with an estimated half of a billion cases worldwide in 2017, which is expected to grow to nearly 700 million by 2045 [2]. Associations between diabetes and cancer have long been observed, with pre-existing diabetes often linked to an increased cancer incidence rate [3]. However, prostate cancer appears to be an exception to this norm, as pre-existing diabetes was shown to be associated with an overall decreased incidence rate [4]. A molecular underpinning to this conundrum remains unclear.

One of the side effects of diabetes is glycation, also known as the Maillard reaction, which is defined as the nonenzymatic glycosylation of macromolecules such as proteins, nucleic acids, and lipids, leading to the formation and accumulation of AGEs. Glycation is known to alter

protein structure, causing amyloid-like aggregation and protein misfolding [5] that can impact cellular proliferation [6] and migration [7], and affect the ECM [8]. In addition, *in vitro* collagen glycation by D-Ribose causes an increase in three-dimensional matrix stiffness and affects endothelial cell behaviour [9]. D-Ribose is a naturally occurring monosaccharide in the human body where it plays a role in ATP production and constitutes a building block for DNA synthesis.

The aim of the present study is to explore the effects of immobilized and soluble AGEs and their receptors in prostate cancer under different matrix stiffness conditions. We found that glycated collagen does not affect cell behaviour, unlike soluble D-Ribose derived BSA AGEs that block phenotypes associated with prostate cancer aggressiveness such as proliferation, spreading and migration. Interestingly, soluble AGEs also blocked stiffness-mediated increase in cell spreading. Additionally, using a publicly available dataset, we noted a differential expression of most of the AGE receptors in prostate tumours, and an association with clinical outcomes. Altogether, our data suggests a protective role for soluble glycated proteins in prostate cancer which could offer a new therapeutic avenue in the treatment of the disease.

2.3 Materials and Methods

Cell culture: All cells were maintained in a humidified incubator at 37℃ with 5% CO_2. PC-3 (CRL-1435™, ATCC), 22Rv1 (CRL-2505™, ATCC), LNCaP (CRL-1740™, ATCC), LAPC4 (kindly provided by Dr. Jacques Lapointe, McGill University), BPH1 (kindly provided by Dr. Axel Thomson, McGill University), and PC-3M-Pro4 (kindly provided by Dr. Mario Chevrette, McGill University) were cultured in Roswell Park Memorial Institute Medium (RPMI) 1640 (350-015-CL, Wisent). DU-145 (HTB-81™, ATCC) and HEK293-FT (kindly provided by Dr. Michel L. Tremblay, McGill University) were cultured in Dulbecco's Modified Eagle Medium (DMEM; 319-015-CL, Wisent). Both media (RPMI and DMEM) were supplemented with heat-inactivated (56℃ for 30 minutes 10% fetal bovine serum (FBS; 12483020, Life Technologies) and 1% HyClone™ penicillin/streptomycin (SV30010, Fisher Scientific). RWPE-1 cells (kindly provided by Dr. Jacques Lapointe, McGill University) were maintained in Keratinocyte-SFM (17005042, Invitrogen) supplemented with recombinant human epidermal growth factor (rEGF) and bovine pituitary extract (BPE) added at the time of use. The MyC-CaP cell line (CRL-

3255™, ATCC), which is a murine cell line established from the Hi-MYC mouse model [10, 11] was maintained in RPMI 1640 with 10% FBS and 1% HyClone™ penicillin/streptomycin.

Collagen glycation: Collagen I was extracted from rat tail tendons (at the Bordeleau laboratory) and glycated by incubating the collagen 10 mg/mL stock (100 µL) with 500 mM D-Ribose (200 µL) (132360250, Acros Organics) in the presence of 1% acetic acid (A38212, Fisher Scientific (700 µL) at 4℃ for 5 days.

AGEs preparation: Fatty acid-free BSA (A4612-25G, Sigma-Aldrich) was dissolved (10 mg/mL) in D-PBS (311-425-CL, Wisent) with or without D-Ribose (Acros Organics) at 100 mM. Both solutions were then filtered and incubated at 37℃ for 3 weeks then stored at 4℃ to be used in experiments.

Spreading and live cell migration assays: For dose response experiments, PC-3, 22Rv1, and MyC-CaP cells were plated on activated glass coverslips coated with 0.1 mg/mL collagen I (Bordeleau laboratory). At the plating time, 100, 200 or 400 µg/mL BSA or BSA-AGEs was added to RPMI with 1%, 2% or 10% FBS. For migration experiments, image acquisition on living cells was performed 3 hours post-plating for 3 hours at 10-minute intervals. For spreading experiments, cells were fixed for 10 minutes at room temperature in 4% paraformaldehyde (PFA) in 1X PBS 15, 30, 60, 120, and 180- minute post-plating. After cell fixation, immunostaining of zyxin (Z4751, Sigma-Aldrich) was performed to evaluate cell area, and DNA was stained with DAPI.

For tunable stiffness experiments, PC-3, 22Rv1 and MyC-CaP cells were plated on 2.5 kPa or 20 kPa polyacrylamide gels coated with 0.1mg/mL collagen I (Bordeleau laboratory) on activated coverslips. At the plating time, 400 µg/mL BSA or BSA-AGEs were added to RPMI 2% FBS. Cells were fixed for 10 minutes at room temperature in 4% PFA in 1X PBS 180-minute post-plating. After cell fixation, immunostaining of zyxin was performed to evaluate cell area, and DNA was stained with DAPI, as described above.

Total distance of cell migration was evaluated with the TrackMate tool of FIJI software (V1.53c). Cell area was also determined with the FIJI software (V1.53c).

Proliferation assays: For the glycated collagen experiments, glycated collagen was diluted in cold D-PBS to a working concentration of 0.1 mg/mL then 150 µL were pipetted into each well of a 96-well plate and incubated for 1 hour at 4℃. Then the excess was aspirated, wells were

washed once with D-PBS and cells were seeded at a 1,000 cells/well density under two different serum conditions (1% and 10%). The plate was then placed in the IncuCyte® S3 (Sartorius) and images of whole wells were acquired every 8 hours, up to 5 days. Images were analyzed using the Basic Analyzer software (included with IncuCyte® system) and data was shown as confluence (%) over time.

For the dose-response experiments, cells were seeded at a density of 1,000 per well (96-well plates). The next day, the culture media was removed and replaced with the appropriate treatment (BSA, BSA-AGEs or D-Ribose) at different concentrations (100, 200 or 400 µg/mL for BSA and BSA-AGEs and 1, 2, or 4 mM for D-Ribose) and serum conditions (1%, 2% or 10%). The plate was then imaged with the IncuCyte® S3 and data was collected and analyzed as previously described.

For the treatment switch experiments, after 3 days, the cell culture media was removed, cells were washed once with nude media, and then the treatment was switched, and the cells were put back in the IncuCyte® S3 and monitored for up to 7 days (post-switch).

Caspase 3/7 cleavage assays: Caspase activity was measured using the IncuCyte® S3 Caspase 3/7 red dye (4704, Sartorius) which is composed of an Asparagine-Glutamine-Valine-Asparagine (DEVD) peptide that can be cleaved by active caspases, a DEVD recognition motif for the binding of the caspases, and a DNA binding dye that emits fluorescence. Cells were seeded at a 2,500 cells/well density in a 96-well plate. The next day, the cell culture media was removed and replaced with appropriate treatment, BSA (400 µg/mL), BSA-AGEs (400 µg/mL) or Etoposide (S1225-100MG, Cedarlane labs) at 5 µM or 10 µM. The IncuCyte® S3 Caspase 3/7 red dye was diluted at 1:500 in FBS-free media and the same media was used to prepare the treatments. The plate was then placed in the IncuCyte® S3 and cells were imaged every 2 hours using the red channel, and red signal was quantified using the Basic Analyzer tool.

Cell cycle analysis: Cells were seeded at 60,000 cells/well density in a 6-well plate. The next day, medium was removed and treatment (BSA or BSA-AGEs at 400 µg/mL) was added for 5 days. Then the treatment was stopped, cells were washed with room temperature D-PBS, detached with trypsin, then counted and pooled (every 3 wells). Cells were then pelleted at 1,500 RPM for 5 minutes, washed again with room temperature D-PBS, then fixed in 70% ethanol for at least 1 hour at 4℃. Afterwards, cells were pelleted again (at 1500 RPM for 5 minutes), then

washed, then stained in a solution containing 10 µg/mL propidium iodide PI (P4170, Sigma-Aldrich), 0.2 mg/mL PureLink RNase A (12091021, Invitrogen), 0.1% Triton X-100 (T8787, Sigma-Aldrich), in D-PBS. Cells were then sorted using flow cytometry with the BD FACSCanto™ system (BD Biosciences) and cell cycle distribution was analyzed using FlowJo software (FLOWJO, LLC v10).

β-galactosidase staining: 11,000 cells were seeded in a 12-well plate, then treated the next day with either BSA or BSA-AGEs (both at 400 µg/mL) for 6 days then β-galactosidase (β-gal) staining was performed using the senescence β-galactosidase staining kit (9860S, Cell Signaling) following the manufacturer's recommendations. Briefly, cells were washed with D-PBS, then fixed with a provided fixing solution, then washed again with D-PBS, then stained with a X-Gal staining solution (prepared from provided reagents), then the plate was wrapped and incubated in a CO_2-free incubator at 37℃ overnight. The next day, cells were imaged under the microscope under 10× magnification and images from different fields were taken for each condition. Positive cells were manually counted, and results were displayed as percentage of total cells. For long-term storage, the staining solution was removed, cells were overlayed with 70% glycerol and the plate was kept at 4℃.

Bioinformatic analyses:

Data source and description: Publicly available prostate cancer transcriptomic datasets were used to analyze AGE receptors expressions and their association with clinical outcomes. TCGA-PRAD readcount matrix and samples clinicopathological information were downloaded from The Cancer Genome Atlas (TCGA) database (http://tcga-data.nci.nih.gov/tcga/) [12] using Bioconductor package TCGAbiolinks [13]. We used TCGA level 3 data comprising of 52 normal and 498 cancer. One metastasis sample was excluded from the analysis.

Data processing: TCGA RNA-seq sequencing read counts were normalized for sequencing depth using the size factor method implemented in Deseq2_1.26.0 package [14].

Survival analysis: Expression data from RNA-seq datasets was transformed using the variance-stabilizing transformation implemented in the Deseq2_1.26.0 package [14]. Patients were divided into high expression and low expression groups by optimal cutpoint calculated by maximally selected rank statistic (surv_cutpoint-function) from the survminer_0.4.6 package [15]. Differences in patient's recurrence-free survival between groups were estimated by

Kaplan–Meier survival analysis and log-rank tests using R package survival_3.1-12 [16] and survival curves were generated using survminer_0.4.6 package [15].
Statistics: All statistical tests were performed in R version 3.6.2 (2019-12-12). Statistical analysis to compare two groups were performed using Wilcox test.

FTIR: Collagen ribose hydrogel (1,5 mg/mL) was dried on a Silicon crystal, then the infrared absorption was measured with an ATR-FTIR (Agilent Cary 660 FTIR, Agilent Technologies, USA), equipped with a deuterated L-alanine-doped triglycine sulfate (DLa-TGS) detector and a Germanium-coated potassium bromide beam splitter. The infrared absorption spectra of untreated collagen hydrogel (1,5 mg/mL) and pure ribose sugar were also acquired as controls.

Spectral analysis: Absorbance at 280 nm was tested with the NanoDrop® spectrophotometer (ND-1000). D-PBS was used as blank to correct for baseline absorbance.

Western Blotting: Increasing quantities of BSA-AGEs (50 ng, 100 ng, 500 ng, 1 µg, 2.5 µg, and 5 µg) were prepared in RIPA buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% TRITON-X), mixed with 4× Laemmli buffer (1610747, Bio-Rad) and boiled at 95℃ for 5 minutes. Samples were then run on a 10% Tris-glycine SDS-polyacrylamide gel and transferred to nitrocellulose blotting membranes (1704271, Bio-Rad), using standard protocols. The membrane was then blocked in 5% milk (made in TBS-T) for 1 hour and probed against AGEs using an anti-CML antibody (MAB3247, R&D) at the recommended concentration (1 µg/mL). Similar protocol was followed for Caspase 3 (9665S, Cell signaling) at 1:1,000 and RAGE (AF1145, R&D) at 1 µg/mL Western Blots where 20 µg and 50 µg of protein were loaded, respectively and GAPDH (2118S, Cell signaling) was used at 1:1,000 as a loading control.

RAGE overexpression: HEK293FT cells were seeded at a 500,000 cells/well density in a 6-well plate. The next day, lipofectamine 2000 (11668019, Invitrogen) was used to transfect 2 wells with a RAGE expression vector (RC204664, OriGene Technologies), 2 wells with H2B-GFP (11680, Addgene) while 2 wells were left non-transfected. 48 hours post transfection, cells were lysed and processed either for Western Blot (see above) or for qPCR. RNA was extracted with QIAzol (79306, Qiagen) and reverse-transcribed into cDNA using the iScript™ Reverse Transcription Supermix (1708841, BioRad) then used for qPCR using the CFX Connect™ Real-Time System (BioRad). ΔΔCt method [17] was used to normalize the RAGE transfected

condition to the non-transfected condition, and GAPDH was used as a housekeeping gene. For h*RAGE* we used the following primer set: forward primer: CACCTTCTCCTGTAGCTTCAGC and reverse primer: AGGAGCTACTGCTCCACCTTCT. As for h*GAPDH* we used the following primer set: forward primer: GTCTCCTCTGACTTCAACAGCG and reverse primer: ACCACCCTGTTGCTGTAGCCAA.

Statistics: Except for **Figure 2-6**, all figures were prepared using GraphPad Prsim 8 software. The Mann-Whitney test was used to compare the distribution between two groups. The Kruskal-Wallis test was used for one-way analysis of variance. Two-way ANOVA was used to compare the mean differences between groups that have been split on two independent variables. Unpaired t-test was used to compare the means of two unmatched groups.

2.4 Results

Immobilized AGEs do not impact prostate cancer cell aggressive phenotypes

We sought to explore the effect of glycated molecules on prostate cancer cells and determine whether phenotypes linked with aggressiveness (such as cell migration, spreading, and proliferation) can be impacted. Glycated entities can either be immobilized (*i.e.,* bound to a given substrate) or soluble *(i.e.,* in suspension). To mimic the former type of AGEs, we opted for glycated collagen for several reasons. First, collagen is the most abundant protein of the ECM, making it physiologically relevant as it is the most common coating substance used in cell culture. Second, collagen glycation has been already characterized by other groups and shown to have an effect on cellular physiology [9]. Third, collagen glycation with D-Ribose is a robust reaction. Collagen glycation was validated by FTIR analysis that showed specific peaks in the glycated condition (**Figure S1**). Glycated collagen did not affect PC-3 migration under three different stiffnesses: 2.5 kPa, which mimics the stiffness of a normal tissue, 20 kPa, which mimics the stiffness of tumours, and glass, which represents a condition of infinite stiffness (**Figure 2-1A**) [18]. Similarly, glycated collagen did not affect PC-3 spreading which increased with the stiffness of the substrate (**Figure 2-1B**). The proliferation of these cells also was not affected by collagen glycation on plastic, under two different serum concentrations (1% and 10%) (**Figure 2-1C**). Similar results were obtained for proliferation using the MyC-CaP murine prostate cancer cell line, under the same conditions (**Figure S2**). These results indicate that collagen glycation does not impact prostate cancer phenotypes, under the tested conditions.

Soluble BSA-AGEs block phenotypes that are associated with cancer aggressiveness in a dose-dependent manner

After seeing no difference with collagen glycation on cellular response, we hypothesized that the accessibility of immobilized AGEs to the cells is compromised with glycated collagen, and we wanted to test whether soluble AGEs in the form of BSA-AGEs would yield a different outcome. Glycation of BSA was validated via Western Blot as increasing quantities of soluble AGEs were detected using an anti-CML antibody (**Figure S3A**). We also performed a spectral absorbance analysis which showed an increase in the absorbance at 280 nm using two different batches of AGEs, compared to non-glycated BSA (**Figure S3B**). Moreover, after the incubation time has passed (three weeks), the soluble AGEs solution turned yellow brown, which can be appreciated with the naked eye, and is used as a colorimetric indicator of a successful glycation reaction, which is also known as a "browning reaction" (**Figure S3C**).

To test the effect of soluble AGEs, we treated the cells with increasing concentrations of BSA-AGEs (100, 200, and 400 μg/mL) prepared in serum-free medium, with the FBS added freshly during the preparation of the treatment at 2%. Non-glycated BSA which has been incubated under the same conditions as the BSA-AGEs was prepared with the same increasing concentrations and used as a control. We noted a dose-dependent decrease in PC-3 live migration, measured by track length (μM) (**Figure 2-2A**). In a similar fashion, PC-3 cell spreading was also decreased in a dose-response manner, measured by cell area (μm^2) (**Figure 2-2B**). As for proliferation, under 2% FBS, we saw a dose-dependent decrease in proliferation, measured by cell confluence (%), upon BSA-AGEs treatment, with a complete blockade in proliferation observed at a concentration of 400 μg/mL (**Figure 2-2C**). Equivalent concentrations of D-Ribose (1, 2, and 4 mM) were diluted in D-PBS and used as an additional control for the proliferation assays. A dose response effect was also observed under 1% FBS (**Figure S4A**) and a decrease in proliferation at 400 μg/mL was still noticed at FBS concentration of 10% (**Figure S4B**). A similar effect on proliferation was seen in the MyC-CaP cell line, at 400 μg/mL, irrespective of the FBS concentration (**Figure S5**). Proliferation was also decreased in a slow-growing human cell line, LAPC4, at 400 μg/mL (**Figure S6**). Interestingly, a dose-response effect on proliferation was observed in a fourth cell line, 22Rv1, under 10% FBS (**Figure S7C**), with no effect seen under lower FBS concentrations, possibly due to the decrease in this cell

line's ability to robustly grow under low serum (**Figures S7A and S7B**). Together, these experiments show that soluble BSA-AGEs block prostate cancer aggressive phenotypes.

Soluble AGEs block stiffness-induced increase in cell spreading

After demonstrating that soluble BSA-AGEs block cell spreading on glass (**Figure 2-2A**) and that cell spreading is increased with stiffness (**Figure 2-1B**), we wanted to investigate whether soluble AGEs could affect the stiffness induction of spreading, by combining AGEs treatment and stiffness modulation in one experimental design. We noted that BSA-AGEs, administered at 400 μg/mL, blunt the increase in cell spreading area that is induced by stiffness in two different prostate cancer cell lines, PC-3 (**Figure 2-3A**), and 22Rv1 (**Figure 2-3B**). This indicates that cellular response to AGEs is maintained, even at high substrate stiffnesses.

The effects of soluble AGEs are irreversible

After the robust decrease in proliferation observed upon AGEs treatment at 400 μg/mL in four different prostate cancer cell lines, we wanted to test whether the cells can recover from an AGEs treatment. To do that, we designed an experiment where we switched the treatment between the control (non glycated BSA) and the experimental (BSA-AGEs) conditions after three days, then monitored cell proliferation for up to 7 days (post-switch). What we noted is that 22Rv1 cells that were initially treated with BSA-AGEs did not recover, despite being treated for 7 days with BSA thereafter (**Figure 2-4**). Importantly, in the other group, BSA-AGEs successfully decreased proliferation, despite a BSA pre-treatment. These results suggest that the effect of soluble AGEs on cell proliferation is irreversible.

AGE-dependent decrease in cellular proliferation is associated with cellular senescence

After noting that the effect of AGEs on proliferation cannot be reversed, we hypothesized that it might be due to apoptosis. We measured caspase 3-7 cleavage using the IncuCyte® S3 red reagent. In 22Rv1, AGEs did not affect apoptotic signal intensity when compared to non-glycated BSA, however etoposide, a DNA-damage inducing drug that is known to cause apoptosis [19], did increase the intensity of red signal in a dose-dependent manner (5 and 10 μM) compared to the vehicle condition (**Figure 2-5A**). Similar results were obtained with the MyC-CaP cell line (**Figure S8A**). In PC-3, Western Blot analysis did not reveal a change in caspase 3 protein levels following AGEs treatment (**Figure S8B**).

We then investigated whether the cell cycle was impacted by AGEs treatment. We saw a significant decrease in the G1 population in 22Rv1 with the treatment (**Figure 2-5B**) coupled with a decrease in cell proliferation, captured by trypan blue cell count (**Figure 2-5C**). Importantly, the cell cycle distribution was not affected in PC-3 by the treatment (**Figure S9A**), whereas proliferation was still decreased as a result of the AGEs treatment (**Figure S9B**).

To assess the effect of the BSA-AGEs treatment on senescence, we used senescence-associated β-gal staining which is accepted as a robust biomarker for senescence in culture and *in vivo* [20]. We noted an increase in β-gal positivity at pH=6 upon AGEs treatment in PC-3 (**Figure 2-6C**). Similar results were obtained with 22Rv1. These results suggest that AGEs treatment is associated with cellular senescence, providing a potential mechanistic explanation to the AGEs-dependent reduction in cellular proliferation.

AGE receptors are differentially expressed in prostate cancer tumours and are associated with clinical outcomes

To determine whether glycation could play a role in prostate cancer disease progression, we examined the gene expression of the nine known AGE receptors in benign prostatic and tumour tissues using the cancer genome atlas (TCGA) dataset which comprised at total 333 primary prostate carcinomas [12]. We found that 78% (7 out of 9) of the AGE receptors are differentially expressed in prostate tumours (**Figure 2-6A**) with some being upregulated in the tumour condition (*DDOST*, *MSR1*, *OLR1*, *PRKCSH*, and *SCARB1*) while the rest is downregulated (*CD36* and *LGALS3*). In prostate cancer, biochemical recurrence (BCR) is defined as the rise in PSA following primary treatment (such as prostatectomy and/or radiation therapy) and is used as a parameter to define clinical outcomes, as shorter BCR time is often indicative of more aggressive disease. We investigated whether this parameter is affected by the expression level of the AGE receptors in this dataset, and we observed that patients bearing prostate tumours with high expression in certain receptors (*AGER*, *MSR1*, *CD36*, *OLR1*, and *STAB1*) experienced biochemical recurrence faster than their low expressing counterparts (**Figure 2-6B)** ($p<0.05$). These results suggest that the AGE receptors are associated with disease development and patient clinical outcomes, and they might potentially be involved in the disease biology.

2.5 Discussion

Despite huge technological advancements made in screening, diagnosis, and treatment, prostate cancer remains one of the most common cancers worldwide and a leading cause of death in men diagnosed with the disease. Therefore, the need for novel treatment modalities that are both safe and efficacious is urgent, due to the presence of factors that increase disease morbidity such as treatment resistance, recurrence, and metastasis.

Our results suggest a protective role for soluble BSA-AGEs in blocking prostate cancer aggressive phenotypes: we note that proliferation is decreased in four different prostate cancer cell lines (three of which are of human origin) upon our treatment. Similarly, the same treatment causes a decrease in cell spreading and migration in the highly metastatic human prostate cancer cell line, PC-3. The literature concerning the role of BSA-AGEs in cellular behavior is problematic, due to conflicting results regarding the way these glycated molecules dictate vital cellular mechanisms such as proliferation and migration. On the one hand, several studies showed that AGEs could block proliferation and migration of cell lines of different origins (both human and non-human) [6, 8] with mechanisms of action only beginning to be elucidated. On the other hand, studies have shown opposite effects, predominantly in the context of cancer, with papers showing that AGEs harbour pro-tumourigenic properties, promoting cell proliferation and invasion *in vitro* [7] and metastasis *in vivo* [21]. Based on that, our results come to add an additional layer of complexity to an already controversial body of knowledge.

There are several important points to keep in mind when studying AGEs in a cellular context, and which can contribute to the opposing results often seen in the literature. First, the type of AGEs matter. As previously alluded to, the Maillard reaction is a chemical reaction that results between the interaction of carbonyl groups of reducing sugars and the amine groups of proteins. Therefore, the members of the AGEs family are numerous and different sugars will undeniably generate different AGEs which could each have different effects on cells. Studies often used different types of AGEs, either “homemade” or commercially purchased, with examples including but not limited to: glycolaldehyde AGEs, methylglyoxal AGEs, glucose derived AGEs, and D-Ribose derived AGEs. Interestingly, a paper that used the same type of AGEs that we use in our own study (*i.e.* D-Ribose derived BSA-AGEs) reached similar conclusions with regards to cell proliferation [5] further corroborating the idea that the glycating agent matters in the context

of AGEs study. Another important aspect of this type of study is the dose. Studies that investigated the effect of AGEs used them in the range of μg/mL with doses ranging between 50 μg/mL and 400 μg/mL. In our study, we opted for a range of 100, 200 and 400 μg/mL for the treatment and we saw a dose-response effect on cell spreading, migration, and proliferation (**Figure 2-2**). Intriguingly, Sharaf *et al.* showed that methylglyoxal BSA-AGEs block breast cancer cell proliferation between 50 and 100 μg/mL, whereas a higher dose (200 μg/mL) does not confer the same effect [7], further confirming that different doses can lead to different outcomes. One other critical aspect when conducting such experiments is controls. Because glycation is a slow and complicated reaction, it is important to thoroughly monitor the preparations for signs of contamination during incubation, which is often conducted at 37℃. Moreover, it is critical to validate the success of glycation, which we did by running our soluble BSA-AGEs on a gel and detecting them by Western Blot using an anti-CML antibody (**Figure S3A**). AGEs are known to form crosslinks, and to polymerize and aggregate, which could explain the presence of multiple bands on the membrane. We were also able to confirm the success of the reaction visually as the BSA-AGEs solution turned yellow brown at the end of the allocated incubation time (3 weeks) (**Figure S3C**) while the non-glycated BSA solution remained transparent. Our glycated collagen has also been validated by FTIR analysis, which showed peaks that are specific to glycation, only in the glycated condition (**Figure S3B**).

The switch experiment (**Figure 2-4**) provides valuable insight into the underlying mechanism of the BSA-AGE effect. We show that 22Rv1 do not recover from the AGE treatment, despite being treated with BSA for 7 days after. Intriguingly, BSA-AGEs are successful in blocking proliferation in this cell line despite a BSA pre-treatment for 3 days. These results indicate that the effects mediated by AGEs are long-lasting and irreversible. However, we show that the treatment does not result in caspases 3 and 7 cleavage, in two different cell lines: 22Rv1 (**Figure 2-5A**) and MyC-CaP (**Figure S8A**), which indicates that AGEs do not cause apoptosis under the tested conditions. Another aspect we wanted to look at was the cell cycle distribution, which showed an increase in the G1 population in 22Rv1 (**Figure 2-5B**) and no effect in PC-3 (**Figure S9A**). Because the phenotype is so similar across cell lines, one would expect the underlying mechanism to be more or less conserved, so it unlikely for the cell cycle distribution to be at least fully responsible for the drastic effects that we see on cell proliferation. Lastly, β-gal staining did increase in the AGEs condition in PC-3 (**Figure 2-5C**) and in 22Rv1, suggestive of

cellular senescence. Senescence provides a plausible explanation for the phenotype that we see, especially since it is irreversible, and offers the exciting possibility of combining AGEs treatment with senolytics to treat prostate cancer.

Although the clinical data presented in **Figure 2-6** is intriguing, it is important to keep in mind that the relationship between RNA expression and protein levels is not necessarily correlational [22]. Therefore, it is not evident that patients who display alterations in the AGERs at the mRNA level will also do so at the protein level, which will be, if involved, mediating the response. In this regard, patient tissue microarrays (TMA) could be used to look directly at the protein and draw correlations with oncological outcomes.

2.6 Conclusion

Altogether, our findings exposed opposite roles for the glycation of soluble proteins and the extracellular matrix stiffness and suggest that the tumour suppressing properties of glycated soluble proteins could be harnessed as a novel therapeutic avenue through precision nutrition. In addition, our results offer a plausible explanation for the protective effect of diabetes against prostate cancer incidence.

However, further investigation is required to uncover the mechanism behind the observed phenotype, which is likely to involve one or more of the AGE receptors. Critically, it is of utmost importance to test the effect of soluble BSA-AGEs *in vivo*, to see whether the protective effect will translate within the context of a more complex biological system.

2.7 Disclosure of potential conflicts of interest

The authors report no potential conflicts of interest.

2.8 Acknowledgments

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2.9 Figures

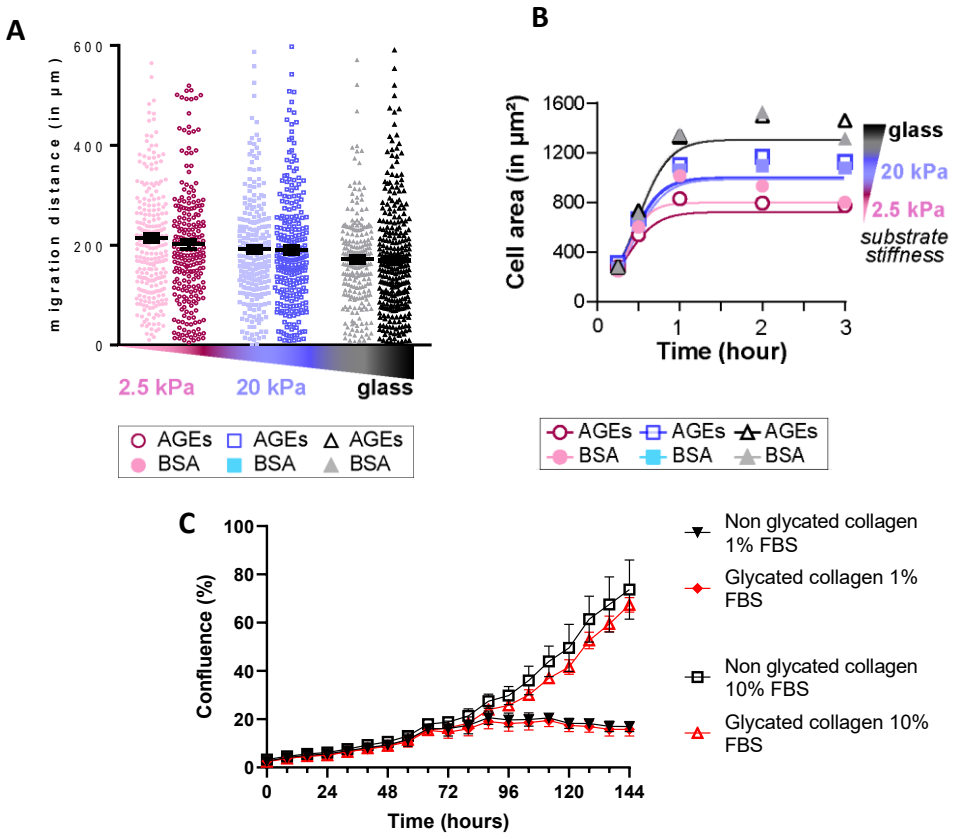


Figure 2-1: Immobilized AGEs do not affect PC-3 aggressive phenotypes.

(A) Glycated collagen does not affect PC-3 migration at any given stiffness. (B) Cell spreading increases with substrate stiffness irrespective of the glycation status. (C) Collagen glycation does not affect PC-3 proliferation. All datapoints from at least two independent experiments are shown for panel (A) as well as the mean ± SEM and an average of at least two independent experiments are shown panels (B,C) ± SD.

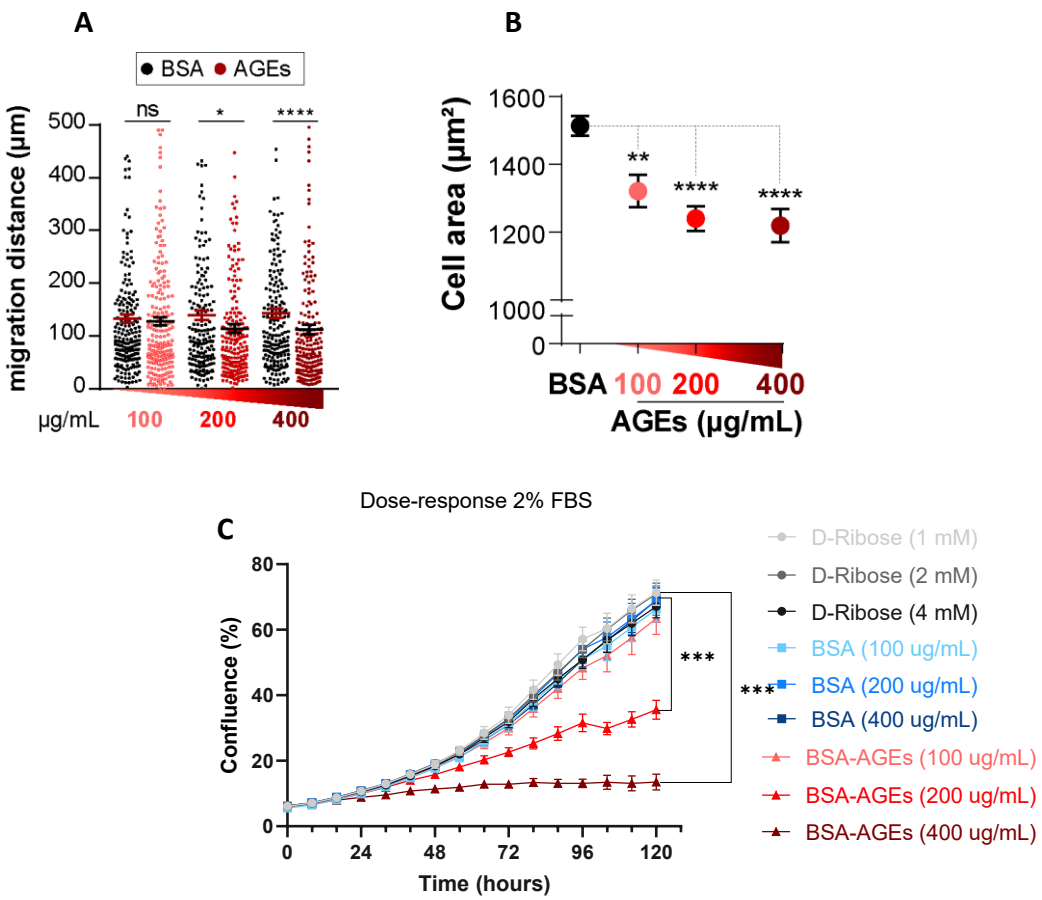


Figure 2-2: Soluble D-Ribose BSA-AGEs block PC-3 aggressive phenotypes.

(A) Live migration is decreased by BSA-AGEs in a dose-dependent manner. All data-points from at least two independent experiments are shown as well as the mean ± SEM. Mann-Whitney test was used to test for significance between BSA and AGEs, at different concentrations. (B) PC-3 cell spreading is decreased by BSA-AGEs in a dose-response manner. Data represents average from at least three independent experiments ± SD. (C) BSA-AGEs block PC-3 proliferation in a dose-dependent fashion. Data represents the average of three independent experiments ± SD; two-way ANOVA was used to test for statistical significance. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

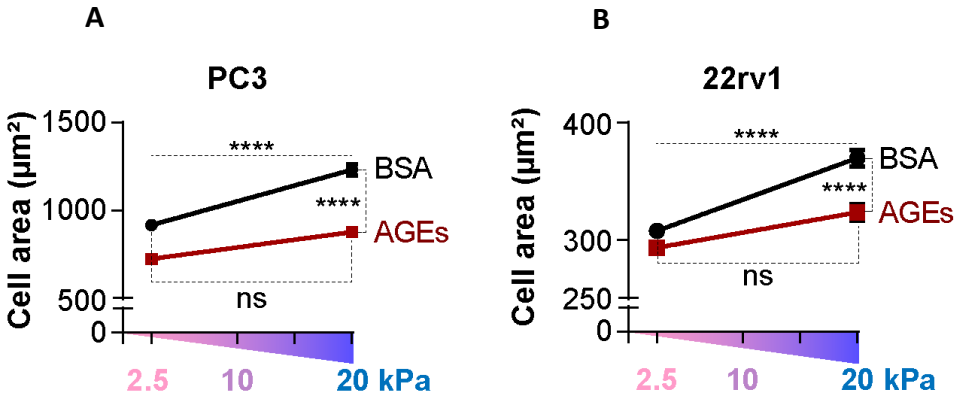


Figure 2-3: Stiffness-induced increase in cell spreading is blocked by soluble BSA-AGEs in two different prostate cancer cell lines.

(A) The effect of BSA-AGEs on PC-3 cell spreading under increasing substrate stiffnesses. (B) The effect of BSA-AGEs on 22Rv1 cell spreading under increasing substrate stiffnesses. Statistical significance was tested by the Kruskal-Wallis one-way analysis of variance. ns > 0.05, **** $p \leq 0.0001$.

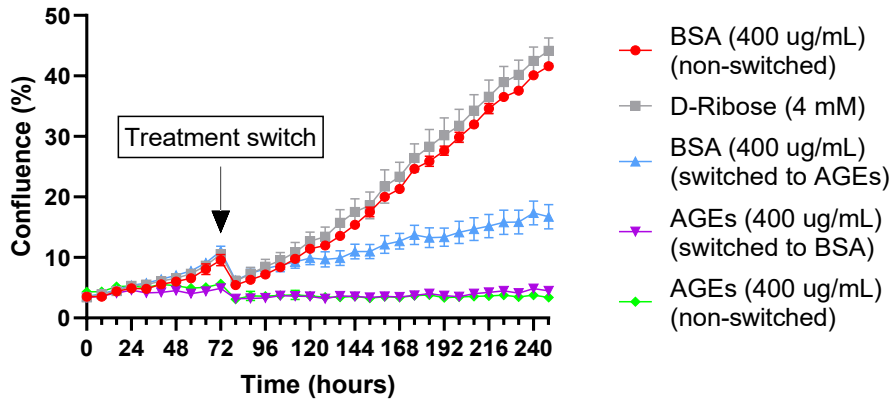


Figure 2-4: Treatment switch does not rescue the proliferation effect mediated by BSA-AGEs in 22Rv1.

Data presented as mean ±SEM from one representative experiment, with four technical replicates per condition. The experiment was repeated twice, and the other experimental replicate is shown in the appendix.

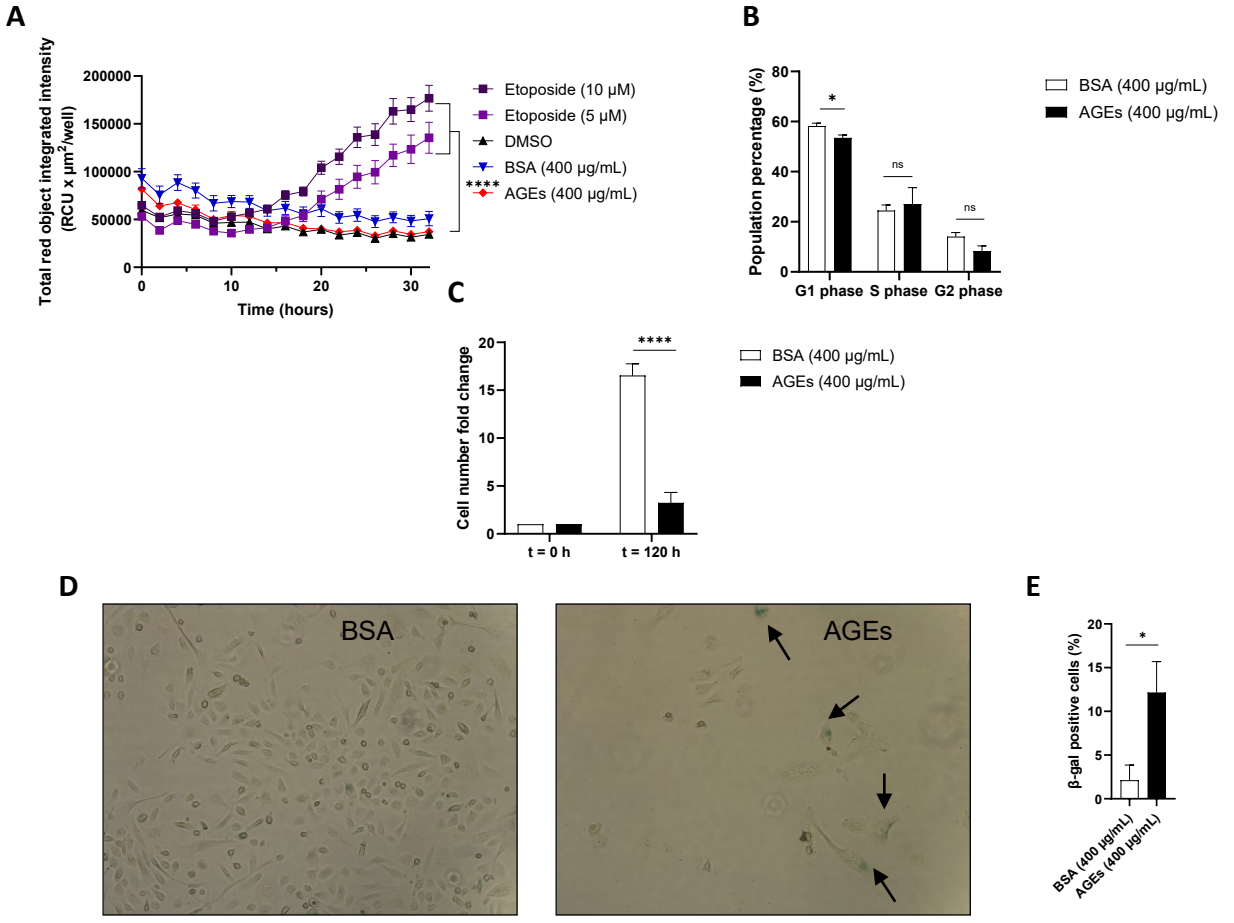


Figure 2-5: BSA-AGEs effect on apoptosis, cell cycle, and senescence.

(A): Soluble BSA-AGEs do not induce apoptosis in 22Rv1. Representative experiment shown (Mean ± SEM; two-way ANOVA). (B) Soluble AGEs cause a decrease in the G1 population in 22Rv1. Average from two independent experiments (Mean ±SEM; unpaired t-test). (C) Decrease in 22Rv1 cell number upon BSA-AGEs treatment. Average from two independent experiments (Mean ±SEM; unpaired t-test). (D) Increase in β-gal staining intensity upon BSA-AGEs treatment in PC-3 (representative images). (E) Quantification of the in β-gal staining from two independent experiments, two different fields per experiment. Data shown as mean ±SEM; unpaired t-test. ns > 0.05, * $p \leq 0.05$, **** $p \leq 0.0001$.

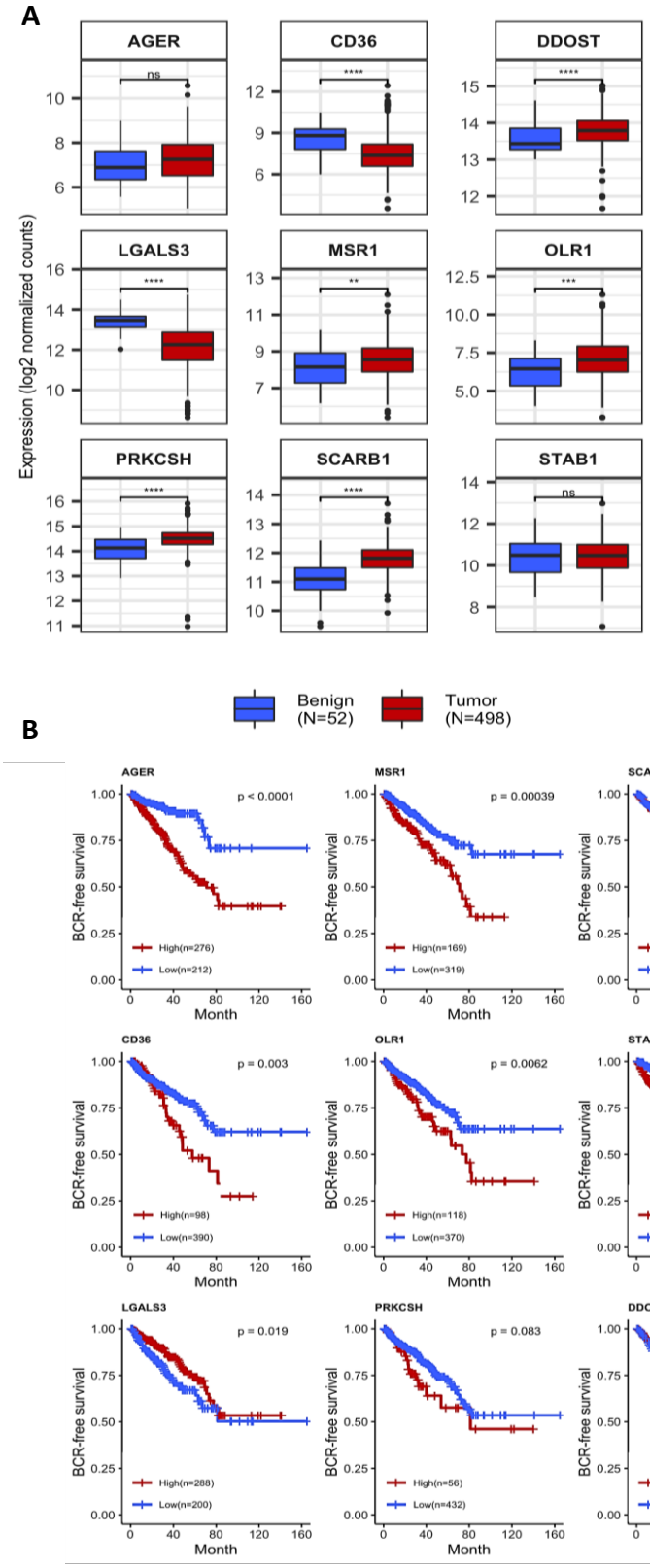


Figure 2-6: AGE receptors are associated with prostate cancer disease progression in the TCGA cohort.

(A) AGE receptors are differentially expressed in prostate tumours. (B) AGE receptors expression level is associated with prostate cancer clinical outcome. Wilcox test. ns $>$ 0.05, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

2.10 Supplementary figures

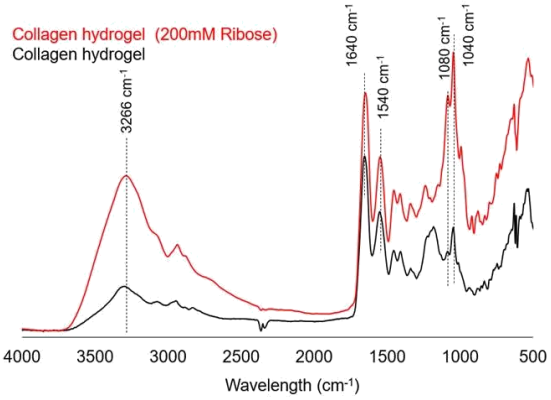


Figure S1: FTIR analysis allows discrimination between non-glycated collagen and glycated collagen hydrogels.

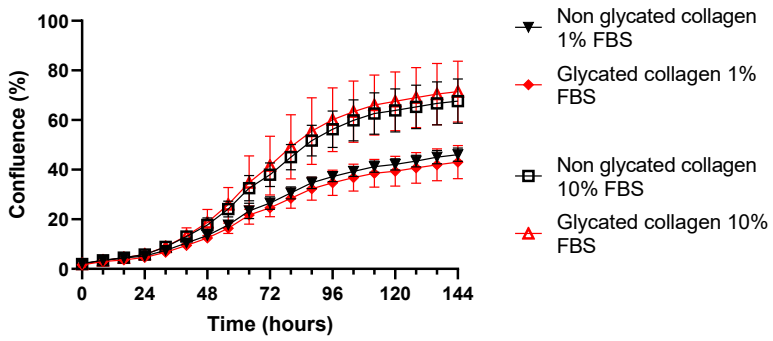


Figure S2: Immobilized AGEs do not have an effect on MyC-CaP proliferation.

Data from two independent experiments (Mean ± SD).

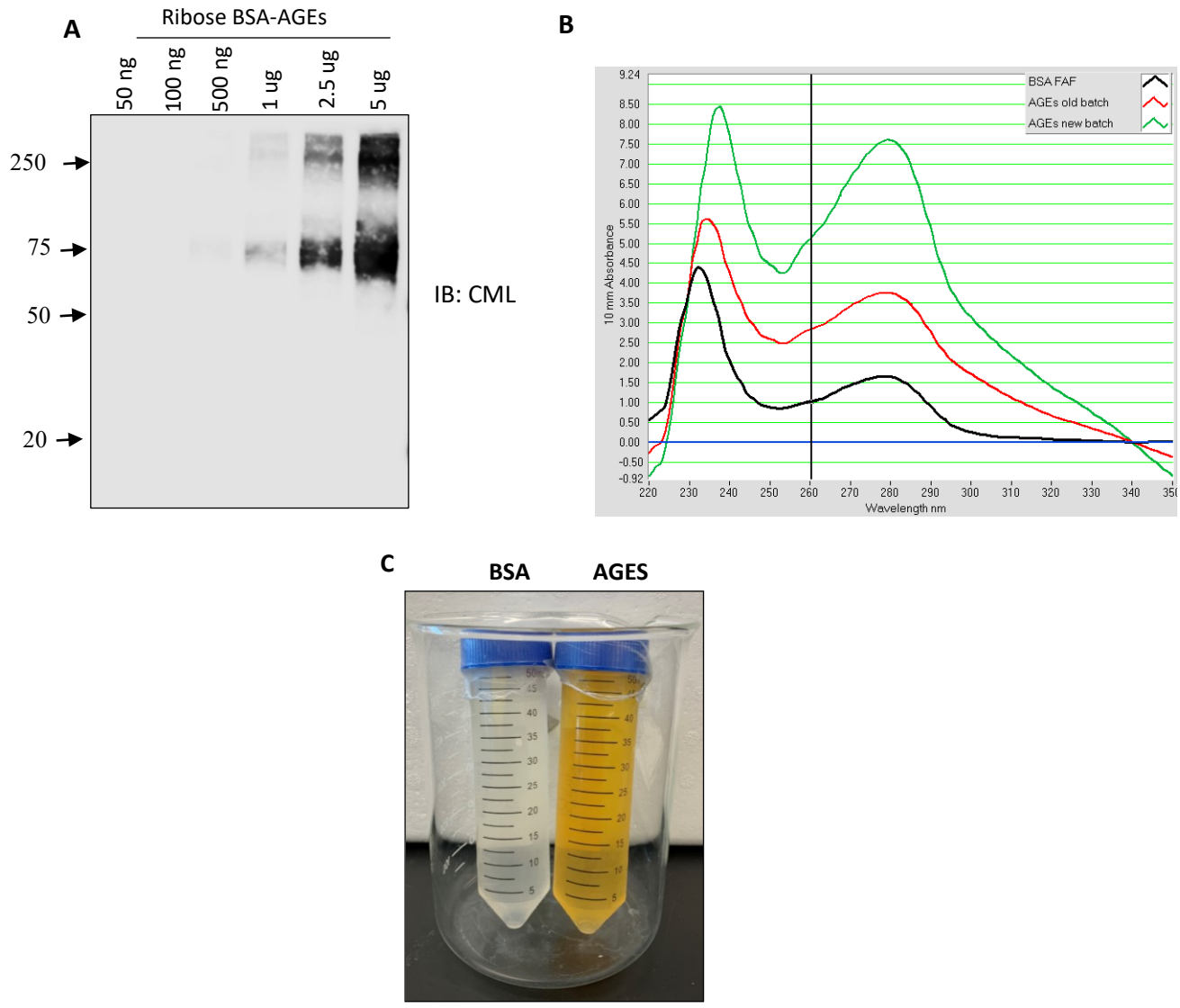


Figure S3: Validation of soluble D-Ribose BSA-AGEs.

(A) Soluble D-Ribose BSA-AGEs are detectable by Western Blot using an anti CML antibody. (B) Soluble D-Ribose BSA-AGEs cause an increase in spectral absorbance at 280 nm. (C) BSA glycation can be appreciated visually by the presence of a yellow-brown solution.

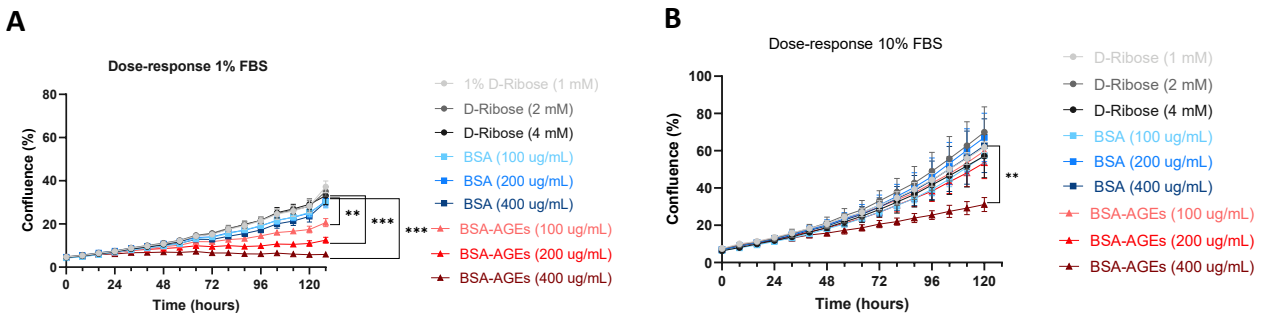


Figure S4: Soluble BSA-AGEs block PC-3 proliferation at different FBS concentrations.

(A) Dose-response effect of soluble BSA-AGEs under 1% FBS. Results from three independent experiments. (B) Soluble AGEs block proliferation at the optimal dose under 10% FBS. Representative experiment shown. Data represents mean ± SD; two-way ANOVA. ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

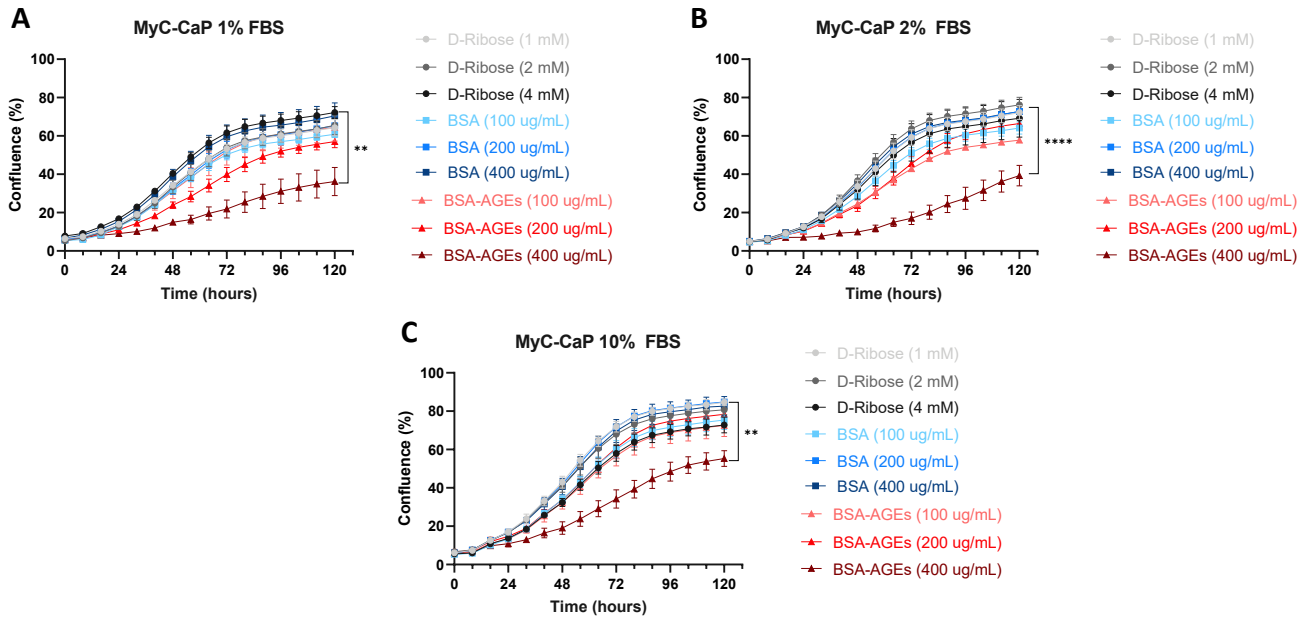


Figure S5: Soluble BSA-AGEs block MyC-CaP proliferation across different FBS concentrations.

1% (A), 2% (B) and 10% (C). Representative experiments shown. Data represents mean ± SD; two-way ANOVA. ** $p \leq 0.01$, **** $p \leq 0.0001$.

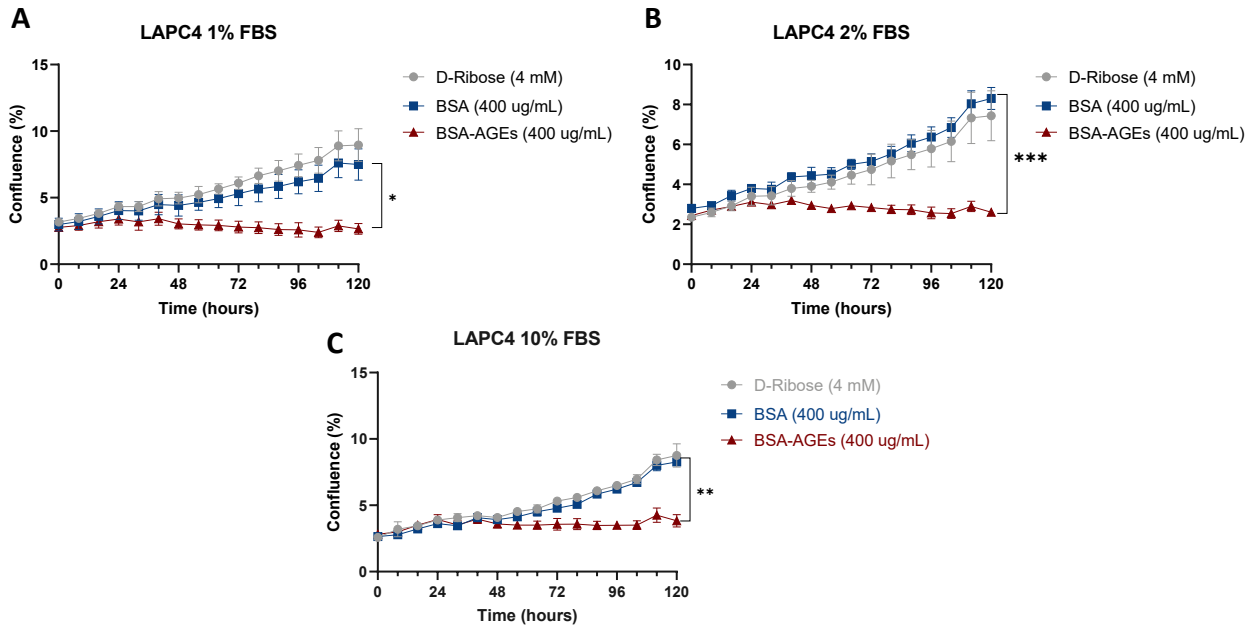


Figure S6: Soluble BSA-AGEs block LAPC4 proliferation across different FBS concentrations.

1% (A), 2% (B) and 10% (C). Representative experiments shown. Data represents mean ± SD; two-way ANOVA. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

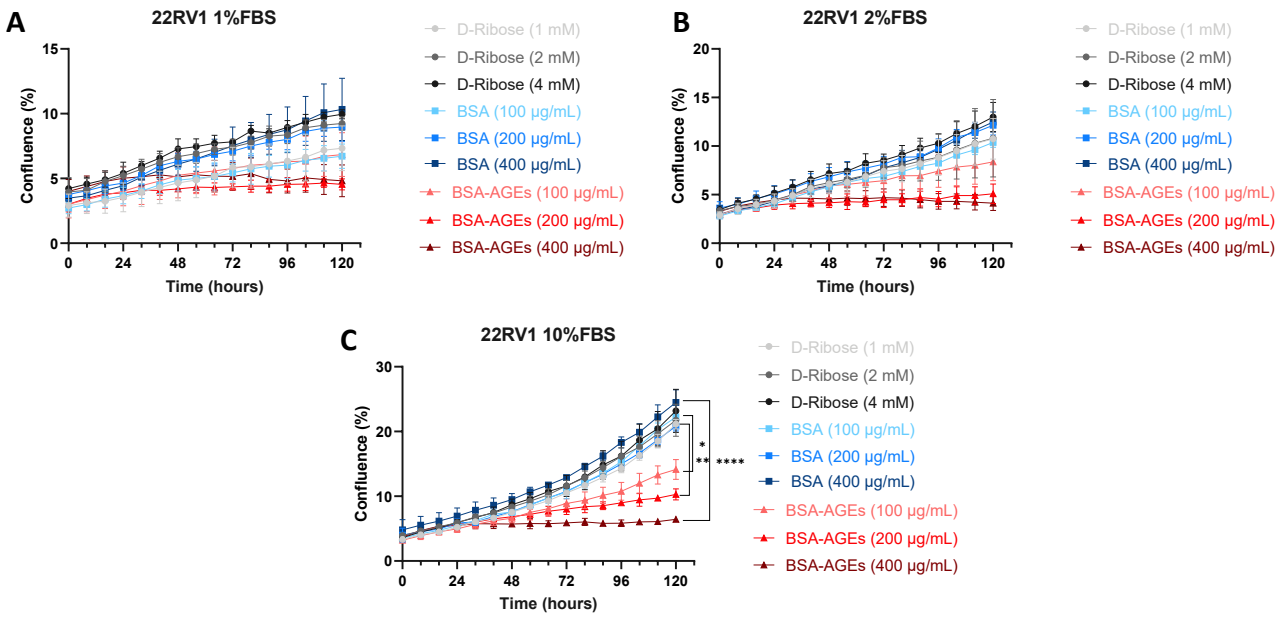


Figure S7: Effect of soluble BSA-AGEs on 22Rv1 proliferation under different FBS conditions.

1% (A), 2% (B) and 10% (C). Representative experiments shown. Data represents mean ± SD; two-way ANOVA. * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$.

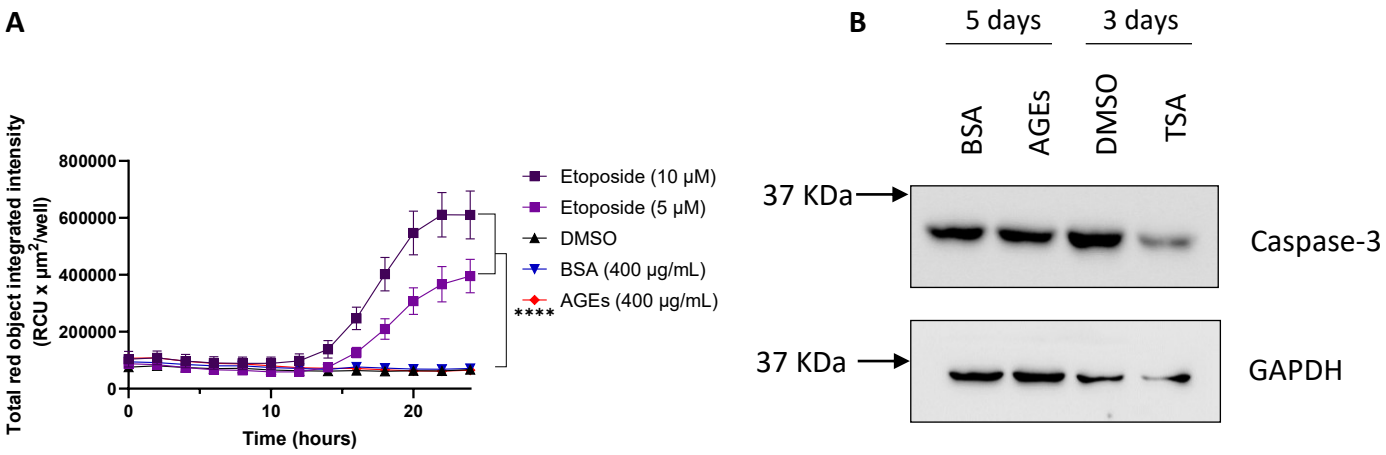


Figure S8: The effects of soluble BSA-AGEs on apoptosis.

(A) Caspase 3-7 cleavage assay in MyC-CaP following BSA-AGEs treatment. Representative experiment shown. Data represents mean ±SEM. (B) Western Blot showing the levels of total Caspase 3 protein in PC-3 cells treated with AGEs. Two-way ANOVA. **** $p \leq 0.0001$.

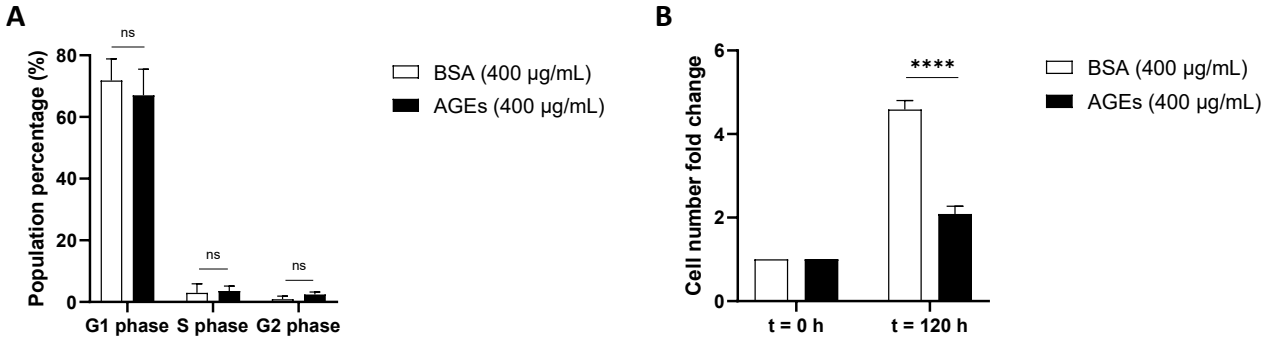


Figure S9: Effect of solube AGEs on PC-3 cell cycle (A) and proliferation (B).

Data from two independent experiments. Mean ± SEM. Unpaired t-test. ns ≥ 0.05,**** p ≤ 0.0001.

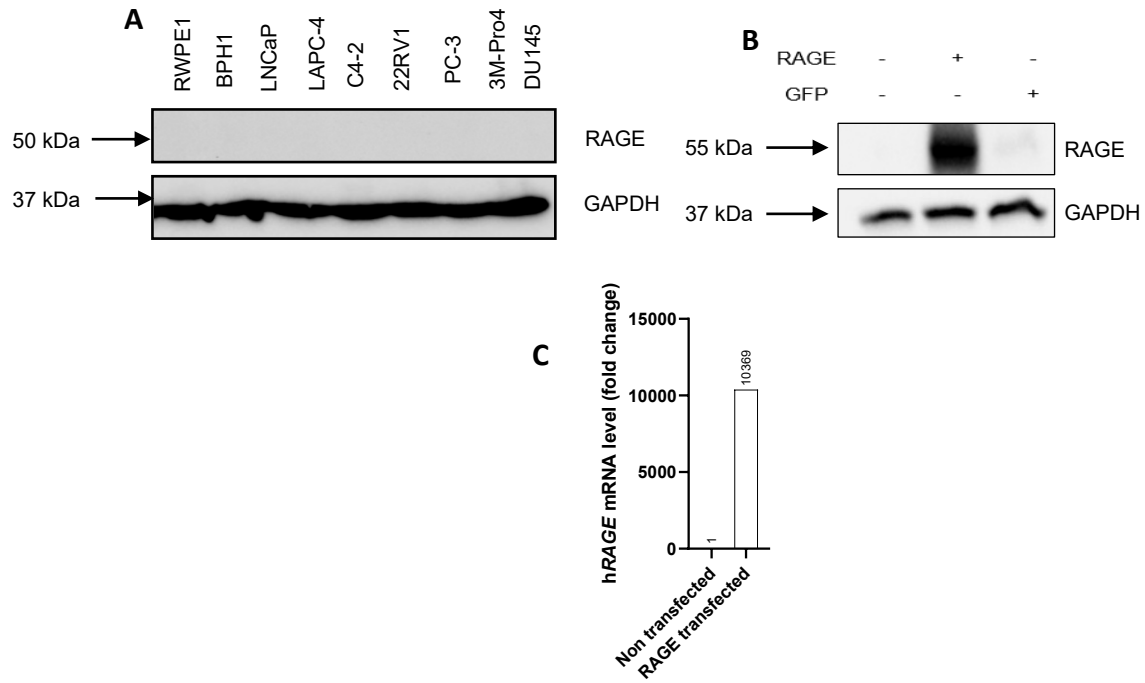


Figure S10: RAGE expression in human prostate cancer cell lines.

(A) RAGE is not expressed in human prostate cancer cell lines at the protein level. (B) The antibody used to detect RAGE in prostate cancer cells is specific. (C) qPCR showing the change in the expression level of *RAGE* following HEK293 transfection.

References for Chapter 2

1. Siegel, R.L., et al., Cancer Statistics, 2021. CA Cancer J Clin, 2021. **71**(1): p. 7-33.
2. Cho, N.H., et al., IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract, 2018. **138**: p. 271-281.
3. Giovannucci, E., et al., Diabetes and cancer: a consensus report. Diabetes Care, 2010. **33**(7): p. 1674-85.
4. Kasper, J.S. and E. Giovannucci, A meta-analysis of diabetes mellitus and the risk of prostate cancer. Cancer Epidemiol Biomarkers Prev, 2006. **15**(11): p. 2056-62.
5. Wei, Y., et al., Rapid glycation with D-ribose induces globular amyloid-like aggregations of BSA with high cytotoxicity to SH-SY5Y cells. BMC Cell Biol, 2009. **10**: p. 10.
6. Li, Y., et al., Advanced Glycation End Products Inhibit the Proliferation of Human Umbilical Vein Endothelial Cells by Inhibiting Cathepsin D. Int J Mol Sci, 2017. **18**(2).
7. Sharaf, H., et al., Advanced glycation endproducts increase proliferation, migration and invasion of the breast cancer cell line MDA-MB-231. Biochim Biophys Acta, 2015. **1852**(3): p. 429-41.
8. Patel, S.H., et al., Advanced Glycation End-Products Suppress Mitochondrial Function and Proliferative Capacity of Achilles Tendon-Derived Fibroblasts. Sci Rep, 2019. **9**(1): p. 12614.
9. Mason, B.N. and C.A. Reinhart-King, Controlling the mechanical properties of three-dimensional matrices via non-enzymatic collagen glycation. Organogenesis, 2013. **9**(2): p. 70-5.
10. Ellwood-Yen, K., et al., Myc-driven murine prostate cancer shares molecular features with human prostate tumors. Cancer Cell, 2003. **4**(3): p. 223-38.
11. Watson, P.A., et al., Context-dependent hormone-refractory progression revealed through characterization of a novel murine prostate cancer cell line. Cancer Res, 2005. **65**(24): p. 11565-71.
12. Cancer Genome Atlas Research, N., The Molecular Taxonomy of Primary Prostate Cancer. Cell, 2015. **163**(4): p. 1011-25.
13. Colaprico, A., et al., TCGAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. Nucleic Acids Res, 2016. **44**(8): p. e71.
14. Love, M.I., W. Huber, and S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol, 2014. **15**(12): p. 550.
15. A, K., Drawing Survival Curves using 'ggplot2'. R package version 0.4.6. http://CRAN.r-project.org/web/packages/survminer. 2016
16. Therneau, T., A Package for Survival Analysis in R. R package version 3.1-12, https://CRAN.R-project.org/package=survival. 2020.
17. Livak, K.J. and T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 2001. **25**(4): p. 402-8.
18. Janmey, P.A., D.A. Fletcher, and C.A. Reinhart-King, Stiffness Sensing by Cells. Physiol Rev, 2020. **100**(2): p. 695-724.
19. Sverchinsky, D.V., et al., Etoposide-Induced Apoptosis in Cancer Cells Can Be Reinforced by an Uncoupled Link between Hsp70 and Caspase-3. International journal of molecular sciences, 2018. **19**(9): p. 2519.

20. Debacq-Chainiaux, F., et al., Protocols to detect senescence-associated beta-galactosidase (SA-βgal) activity, a biomarker of senescent cells in culture and in vivo. Nature Protocols, 2009. **4**(12): p. 1798-1806.
21. Deng, R., et al., Glucose-derived AGEs promote migration and invasion of colorectal cancer by up-regulating Sp1 expression. Biochim Biophys Acta Gen Subj, 2017. **1861**(5 Pt A): p. 1065-1074.
22. Koussounadis, A., et al., Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. Scientific Reports, 2015. **5**(1): p. 10775.

Chapter 3: General discussion

3.1 Summary of findings

Throughout this thesis, we demonstrated a potential protective role for soluble BSA-AGEs in blocking or decreasing prostate cancer aggressive phenotypes *in vitro* using different human prostate cancer cell lines (PC-3, 22Rv1, and LAPC4) and one *MYC* overexpressing murine cell line (MyC-CaP). We studied several key aspects of tumour cell biology such as proliferation, cell spreading, and migration and found that all of these mechanisms are intriguingly interrupted upon soluble BSA-AGEs treatment. We show that the observed phenotype is not mediated by classical cell death pathway (under the tested conditions) nor defects in cell cycle distribution. We also show that the effect might be due to an increase in cellular senescence, however this requires further characterization, which will be discussed in this chapter. Intriguingly, we note that immobilized AGEs, in the form of glycated collagen, do not exert any effect on the mechanisms that we tested, for reasons that will be further discussed. Additionally, we noted a blockade of stiffness-induced spreading and migration by soluble AGEs, hinting at a potential crosstalk between soluble BSA-AGEs and matrix stiffness.

3.2 The impact of soluble BSA-AGEs on prostate cancer cell proliferation

Cell proliferation, *i.e.,* the cell's ability to increase in number and divide, is at the core of cancer growth and progression. In fact, in very simple terms, cancer is nothing but unregulated proliferation of tumour cells [160]. Therefore, it is imperative to find agents that can regulate cancer cell proliferation, offering new potential therapeutic avenues. In our study, we used four different cell lines, harbouring different genetic alterations and androgen receptor status to have a better representation of the disease: for instance, PC-3 and 22Rv1 could represent an advanced stage of the disease, one that is metastatic and androgen insensitive while LAPC4 cells represent earlier stages. As for the MyC-CaP cell line, despite being a murine model, it is an indispensable tool to study the disease in a *MYC*-driven prostate cancer context, especially since it has been demonstrated to be particularly vulnerable to metabolic intervention [44]. Live imaging was favoured over more traditional methods to quantify proliferation due to its feasibility, its ease to streamline, and the ability to monitor the experiment for longer periods of time. We used different FBS concentrations (1%, 2%, and 10%) to mimic the nutrient gradient within the

tumour microenvironment. The fact that soluble BSA-AGEs block proliferation at the high dose (400 μg/mL) under both low (1%) and high (10%) FBS concentrations indicates that this treatment impacts cancer cells biology irrespective of nutrient availability, which could have major implications in clinical settings as most cancer cells rely on altered energy metabolism to fuel their malignancy [22].

3.3 The effect of soluble BSA-AGEs on prostate cancer cell spreading and migration

In addition to proliferation, cell spreading and migration are two physiological phenomena with some common features, and they both can affect cancer cellular biology. Cell spreading is defined as the cell's ability to flatten on a surface in order to adhere to a substrate. It is a passive phenomenon, at least in the early stages, and it has significant implications on other cellular functions such as proliferation, motility, and fate determination [161]. Cell migration, which is defined as a cell's ability to move from one location to another, is crucial for both physiological and pathological processes. Study of cell migration in the context of cancer biology is of particular interest because metastasis, which is the feature that makes cancer so deadly, is thought to be mediated by cancer cell motility and migration [162]. Our results showed that, in a similar fashion to proliferation, a treatment with BSA-AGEs affect cell spreading and migration. In the highly metastatic PC-3 cell line, the treatment was shown to decrease cell migration, measured by track length (μm) in a dose-response fashion (**Figure 2-2A**). The same trend is observed with cell spreading (**Figure 2-2B**), measured by cell area (μm^2). Since these cellular processes are interconnected and known to influence one another, the effect of the soluble BSA-AGEs being similar across different processes is not surprising and a sequence of events where cells treated are unable to properly spread on the matrix surface which impairs their ability to proliferate and migrate is highly plausible.

3.4 Crosstalk between soluble BSA-AGEs and matrix stiffness: soluble AGEs as a regulatory mechanism

Matrix stiffness is known to affect cellular physiology, with regards to proliferation, adhesion and even cell fate determination [163]. For cell spreading, we decided to tune the substrate stiffness to mimic a more physiologically relevant state where cells are bound to softer substrates (2.5 – 20 KPa) and we saw that AGEs blunt the effect of the stiffness on spreading, in two different human cell lines (PC-3 and 22Rv1) (**Figure 2-3**). This result suggests that the

phenotypes observed with soluble BSA-AGEs could be a part of a regulatory mechanism that maintains tissue homeostasis under conditions of high glycation: in this context, the increase in matrix stiffness, which would drive cell spreading, is counterbalanced by the increase in the concentration of soluble BSA-AGEs in circulation, to block the tumour-promoting effects of stiffness.

3.5 The effect of soluble BSA-AGE on senescence: the use of senolytics in cancer treatment

The most striking difference between senescence and other types of quiescence is that the former is irreversible while quiescent cells can restore their proliferative capacity once the growth conditions are favourable again [164]. Our results indicate clearly that when cells are treated with BSA-AGEs, they become quiescent (*i.e.,* they stop proliferating). Moreover, in the 22Rv1 cell line, changing the condition from BSA-AGEs to BSA 3 days after the initial treatment does not trigger the cells to proliferate again, despite the favourable condition being restored (BSA), which indicates that the effects of soluble BSA-AGEs are irreversible and long-lasting. We do show that the effect is not due to caspase 3-7 mediated apoptosis (**Figure 2-5A**) nor defects in cell cycle distribution (**Figure 2-5B**). However, our results show an increase in β-gal staining intensity in PC-3 (**Figure 2-5D**), indicating that the BSA-AGEs treatment might be causing the cells to become senescent.

Senolytics are defined as an emerging class of drugs that can selectively clear senescent cells *in vitro* and *in vivo* [165]. Evidence from animal models show that the use of senolytics could promote longevity in animals, as well as having beneficial effects on several diseases such as atherosclerosis, renal dysfunction, and cancer [166]. While still in the preclinical stages, senolytics represent an exciting new therapeutic avenue in cancer where therapy induced senescence (TIS), caused by the use of classical antineoplastic treatments such as radiotherapy and chemotherapy, is harnessed to selectively target and clear senescent tumour cells, which could delay or even prevent cancer relapse [167]. In our model, it would be exciting to combine the effect of soluble BSA-AGEs and senolytic drugs and assess how this combination could play out in the context of tumour growth *in vivo*. If the effect of BSA-AGEs on cancer cell proliferation is conserved *in vivo*, which is yet to be determined, and if BSA-AGEs are indeed causing senescence, one would expect the combination of both treatments to slow tumour growth

in mice or inhibit tumour initiation altogether. The answer to these two different concepts relies in timing of both treatments in respect to the time of injection of tumour cells.

3.6 Prostate cancer cell response to immobilized AGEs: the effect of glycated collagen

Immobilized AGEs, administered in the form of glycated collagen did not influence prostate cancer phenotypes (**Figure 2-1**) and this could be due to several reasons. Despite glycation being validated by FTIR, we are not able to quantify the level of glycation and thus determine whether it is sufficient to induce a change in cellular response. Our collagen has been glycated for 5 days only, and even though this condition has been proven to be sufficient to affect endothelial cells [168], it is yet to be determined whether longer incubation times would yield different outcomes in our system. One way to address this question would be by testing different incubation times, ranging from the current one (5 days) and going up to several weeks, then systemically comparing them within the same experiment. One issue that could arise, however, is that longer incubation times might cause significant changes in collagen structure and architecture [169], making it difficult to decouple the AGEs *per se* from the collagen structure as the culprit for any possible observed change in cell response. One other plausible explanation for the lack of effect on prostate cells is accessibility. Immobile AGEs, which are bound to the collagen that is used to coat the plates on which cells are seeded may be less accessible to these cells, especially when compared to soluble AGEs. Because the effect of AGEs is most likely to be mediated by at least one of the AGE receptors, a ligand-receptor interaction is necessary to provoke a response. And because receptors that bind ligands of the ECM generally do so with low affinity, it is tempting to speculate that a potential low binding capacity of AGE receptors might be in part to blame for the lack of response.

3.7 Physiological relevance of the results in the context of diabetes

Glycation, and the subsequent formation of AGEs, are hallmarks of diabetes mellitus, with the use of glycated hemoglobin as a means to monitor long-term glycemic levels (as alluded to in **Chapter 1**). In fact, glycation has been suggested as a mechanism to bridge hyperglycemia and health complications observed in diabetic patients [170]. Our study investigated the role of soluble BSA-AGEs which are derived from the reaction between D-Ribose and BSA. Intriguingly, circulating glycated serum albumin has been found to be elevated in the blood of

diabetic patients [171], adding a layer of clinical relevance to our findings. Although D-Glucose is one of the most important players in diabetes, there has been no direct evidence linking higher glucose levels to the increase in glycated serum [172]. On the other hand, D-Ribose was found to be increased in the urine of diabetic patients [173]. D-Ribose was also shown to be involved in the glycation of hemoglobin [174] and auto antibodies against D-Ribose glycated hemoglobin were detected in diabetic patients [175]. This suggests that D-Ribose, often overlooked in the context of diabetes, might be mediating many diabetes-related health complications, and that our results could be potentially relevant for diabetic patients. The demonstrated effect of soluble BSA-AGEs on prostate cancer cells in our study could serve as a mechanistic explanation for the paradoxical decrease in prostate cancer incidence observed in diabetic patients [176].

3.8 Precision nutrition in cancer: is there a room for AGEs?

Evidence from epidemiological and clinical studies have long established a relationship between diet and cancer [177], with certain diets (such as the Western Diet) linked to an increase in cancer incidence while others (such as the Mediterranean diet) are associated with a decrease in cancer incidence [178]. Moreover, certain food groups have been found to harbour cancer protective properties, such as fruits and vegetables [179]. Theories to explain the cancer-diet relationship range from immune modulation to changes in the gut microbiome, as well as direct effects on cancer cells metabolism [180]. One limitation, however, to the entire cancer and diet discourse is the inability to decouple the effect of certain micronutrients from the effects of macronutrients, which makes the application of dietary intervention in cancer somehow controversial. Nevertheless, dietary intervention is emerging as an exciting therapeutic avenue where the antitumour properties of certain food molecules or dietary patterns can be harnessed to boost the efficacy of current cancer treatments, although clinical trials in that regard are still in their infancy [181]. The way AGEs could fit within this puzzle is tricky due to the conflicting literature surrounding these molecules and their effect on cancer. Moreover, it is important to distinguish between exogenous or dietary AGEs, which are obtained through the diet, mainly by consuming certain foods (red meat, fried potatoes, etc.), and endogenous AGEs, which are produced by the Maillard reaction inside the body. Currently, there is no convincing evidence linking consumption of exogenous AGEs to the level of circulating endogenous AGEs [80]. Intriguingly, a follow-up study found an inverse association between fructosamine (a glycated protein) serum levels and prostate cancer incidence risk [182], which is in congruent with our

own findings. It is still unclear how AGEs could be potentially employed in the context of dietary intervention as our model is still at the preclinical level, however control of glycated serum protein levels might prove useful in prostate cancer prevention and treatment.

3.9 Human data: the AGE receptors and how they could be mediating the phenotype

The AGE receptors constitute an important piece of the puzzle that is not to be overlooked. Bioinformatic analysis of a publicly available dataset revealed that some of the receptors are differentially expressed in prostate tumours (**Figure 2-6A**). In addition, the expression of some of these receptors seems to be associated with clinical outcomes. It is important to keep in mind that association does not necessarily mean causation. However, these data provide clinical relevance to our study which has only used *in vitro* models, so far. Intriguingly, in our system, RAGE, which is the most studied AGE receptor, was not expressed at the protein level (**Figure S10A**) in human prostate cancer cell lines that we looked at. The antibody for RAGE was promptly validated by overexpression at the level of the protein (**Figure S10B**) and the mRNA (**Figure S10C**). Therefore, it is unlikely for RAGE to be mediating the effects that we see in our experiments, since it is not expressed in the human cell lines that we used. The scenario where other receptors are involved is possible. One way to address this would be by systemically knocking out the receptors, one by one, then treating cells with BSA-AGEs, and assessing whether the proliferation phenotype can be rescued, which would help identify one of the receptors as a mediator, if the effect is indeed receptor-mediated.

Chapter 4

General conclusions and future directions

4.1 General conclusions

The main aim of this thesis was to explore the role of glycation, in the form of immobilized AGEs or soluble serum proteins, in prostate cancer cell biology. Diabetes, a known catalyst of glycation in humans, is a disease that affects millions of people worldwide and is known to impact cancer incidence and outcomes [71].

We investigated phenotypes that are tightly related to tumour aggressiveness such as proliferation, migration, and spreading and noted, for the first time, a protective effect for soluble glycated serum proteins in prostate cancer. Our findings were consistent across several cell lines of different origins, underlining the impact of our results across multiple prostate cancer disease stages and in the context of various genetic alterations.

The implications for the results presented in this thesis are double-sided. On one hand, the tumour suppressive properties of soluble AGEs might serve as a molecular underpinning to the protective effect of diabetes against prostate cancer incidence. On the other hand, while still at the preclinical stage, these results open the door for the use of soluble glycated proteins in cancer treatment, in the context of precision nutrition.

4.2 Future directions

The results presented in this study are highly encouraging in respect to cancer treatment and prevention. However, further characterization is definitely required in order to fully understand the mechanism through which soluble BSA-AGEs block prostate cancer cellular phenotypes.

First, it is critical to determine the molecular landscape that is underlying the effect that we observe with the soluble BSA-AGEs treatment. Because the phenotype is very robust, we expect significant changes at the level of gene expression following the treatment. In that regard, RNA-seq will prove a powerful tool to pinpoint specific pathways that are mediating the response which can then dictate future experiments to target said pathways. We expect to define what we call an “AGEs-dependent signature” that will help expose the molecular maestro that is orchestrating the AGE-mediated cellular response.

Second, it is important to study the AGEs response in the context of AGERs. This is one caveat to our study since in our model, RAGE, which is the most studied and characterized AGE receptor, was not detectable at the protein nor mRNA levels. Nevertheless, we cannot eliminate the possibility of a ligand-receptor interaction underlying the effect. To test that, the different AGERs need to be molecularly altered by knockout and cells then treated with AGEs, which could help uncover one or more receptors as culprits.

Third, we must assess how our results might play out *in vivo*, within a more intricate biological entity, especially in the context of TME. The fact that our experiments involved both human and murine cell lines mean that we can study the effect of AGEs in both prostate cancer xenograft and allograft models, which will allow us to investigate the ramifications of the treatment in the presence of a functional immune system, offering a better representation of the conditions in human disease.

Fourth, it would be interesting to look at certain keys of our research project in a clinical human setting. Patient derived TMA could be useful in that regard. For example, we could look at the expression of the AGE receptors in tumour tissues and correlate it with patient outcomes. Additionally, we can probe against glycated molecules within those tissues and assess their prevalence. This will undeniably add a layer of clinical relevance to our study that is still currently at the preclinical stage.

In summary, *in vitro*, *in vivo*, *in silico*, and clinical tools should be harnessed to draw a bigger and a clearer picture of how glycated molecules impact prostate cancer development and progression.

References for Chapters 1, 3 and 4

1. Brenner, D.R., et al., *Projected estimates of cancer in Canada in 2020.* CMAJ, 2020. **192**(9): p. E199-E205.
2. Packer, J.R. and N.J. Maitland, *The molecular and cellular origin of human prostate cancer.* Biochim Biophys Acta, 2016. **1863**(6 Pt A): p. 1238-60.
3. El-Alfy, M., et al., *Unique features of the basal cells of human prostate epithelium.* Microsc Res Tech, 2000. **51**(5): p. 436-46.
4. Zhang, D., et al., *Prostate Luminal Progenitor Cells in Development and Cancer.* Trends Cancer, 2018. **4**(11): p. 769-783.
5. Kim, M.J., et al., *Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis.* Proc Natl Acad Sci U S A, 2002. **99**(5): p. 2884-9.
6. Ellwood-Yen, K., et al., *Myc-driven murine prostate cancer shares molecular features with human prostate tumors.* Cancer Cell, 2003. **4**(3): p. 223-38.
7. Greenberg, N.M., et al., *Prostate cancer in a transgenic mouse.* Proc Natl Acad Sci U S A, 1995. **92**(8): p. 3439-43.
8. Wang, Z.A., et al., *Luminal cells are favored as the cell of origin for prostate cancer.* Cell Rep, 2014. **8**(5): p. 1339-46.
9. Goldstein, A.S., et al., *Identification of a cell of origin for human prostate cancer.* Science, 2010. **329**(5991): p. 568-71.
10. Bonnet, D. and J.E. Dick, *Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell.* Nat Med, 1997. **3**(7): p. 730-7.
11. Collins, A.T., et al., *Prospective identification of tumorigenic prostate cancer stem cells.* Cancer Res, 2005. **65**(23): p. 10946-51.
12. Li, S., et al., *Chemosensitization of prostate cancer stem cells in mice by angiogenin and plexin-B2 inhibitors.* Commun Biol, 2020. **3**(1): p. 26.
13. Dubrovska, A., et al., *The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations.* Proc Natl Acad Sci U S A, 2009. **106**(1): p. 268-73.
14. Cojoc, M., et al., *Aldehyde Dehydrogenase Is Regulated by beta-Catenin/TCF and Promotes Radioresistance in Prostate Cancer Progenitor Cells.* Cancer Res, 2015. **75**(7): p. 1482-94.
15. Lawrence, M.S., et al., *Discovery and saturation analysis of cancer genes across 21 tumour types.* Nature, 2014. **505**(7484): p. 495-501.
16. Jenkins, R.B., et al., *Detection of c-myc oncogene amplification and chromosomal anomalies in metastatic prostatic carcinoma by fluorescence in situ hybridization.* Cancer Res, 1997. **57**(3): p. 524-31.
17. Barbieri, C.E., et al., *The mutational landscape of prostate cancer.* Eur Urol, 2013. **64**(4): p. 567-76.
18. He, W.W., et al., *A novel human prostate-specific, androgen-regulated homeobox gene (NKX3.1) that maps to 8p21, a region frequently deleted in prostate cancer.* Genomics, 1997. **43**(1): p. 69-77.
19. Abdulkadir, S.A., et al., *Conditional loss of Nkx3.1 in adult mice induces prostatic intraepithelial neoplasia.* Mol Cell Biol, 2002. **22**(5): p. 1495-503.
20. Bostwick, D.G., D. Ramnani, and J. Qian, *Prostatic intraepithelial neoplasia: animal models 2000.* Prostate, 2000. **43**(4): p. 286-94.

21. Robinson, D., et al., *Integrative Clinical Genomics of Advanced Prostate Cancer.* Cell, 2015. **162**(2): p. 454.
22. Hanahan, D. and R.A. Weinberg, *Hallmarks of cancer: the next generation.* Cell, 2011. **144**(5): p. 646-74.
23. Cheng, I., et al., *Prostatitis, sexually transmitted diseases, and prostate cancer: the California Men's Health Study.* PLoS One, 2010. **5**(1): p. e8736.
24. Patel, A.R. and E.A. Klein, *Risk factors for prostate cancer.* Nat Clin Pract Urol, 2009. **6**(2): p. 87-95.
25. Grozescu, T. and F. Popa, *Prostate cancer between prognosis and adequate/proper therapy.* J Med Life, 2017. **10**(1): p. 5-12.
26. Bashir, M.N., *Epidemiology of Prostate Cancer.* Asian Pac J Cancer Prev, 2015. **16**(13): p. 5137-41.
27. Sakr, W.A., et al., *The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients.* J Urol, 1993. **150**(2 Pt 1): p. 379-85.
28. C, J., et al., *"More men die with prostate cancer than because of it" - an old adage that still holds true in the 21st century.* Cancer Treat Res Commun, 2021. **26**: p. 100225.
29. Shenoy, D., et al., *Do African-American men need separate prostate cancer screening guidelines?* BMC Urol, 2016. **16**(1): p. 19.
30. Rebbeck, T.R., *Prostate Cancer Genetics: Variation by Race, Ethnicity, and Geography.* Semin Radiat Oncol, 2017. **27**(1): p. 3-10.
31. Lachance, J., et al., *Genetic Hitchhiking and Population Bottlenecks Contribute to Prostate Cancer Disparities in Men of African Descent.* Cancer Res, 2018. **78**(9): p. 2432-2443.
32. Coughlin, S.S., *A review of social determinants of prostate cancer risk, stage, and survival.* Prostate Int, 2020. **8**(2): p. 49-54.
33. Halbert, C.H., et al., *Racial differences in medical mistrust among men diagnosed with prostate cancer.* Cancer, 2009. **115**(11): p. 2553-61.
34. Ren, Z.J., et al., *First-degree family history of breast cancer is associated with prostate cancer risk: a systematic review and meta-analysis.* BMC Cancer, 2019. **19**(1): p. 871.
35. Klein, E.A., P.A. Kupelian, and J.S. Witte, *Does a family history of prostate cancer result in more aggressive disease?* Prostate Cancer Prostatic Dis, 1998. **1**(6): p. 297-300.
36. Gillanders, E.M., et al., *Combined genome-wide scan for prostate cancer susceptibility genes.* J Natl Cancer Inst, 2004. **96**(16): p. 1240-7.
37. Giri, V.N. and J.L. Beebe-Dimmer, *Familial prostate cancer.* Semin Oncol, 2016. **43**(5): p. 560-565.
38. Zheng, S.L., et al., *Cumulative association of five genetic variants with prostate cancer.* N Engl J Med, 2008. **358**(9): p. 910-9.
39. Ellinger, J., et al., *CpG island hypermethylation at multiple gene sites in diagnosis and prognosis of prostate cancer.* Urology, 2008. **71**(1): p. 161-7.
40. Nowacka-Zawisza, M. and E. Wisnik, *DNA methylation and histone modifications as epigenetic regulation in prostate cancer (Review).* Oncol Rep, 2017. **38**(5): p. 2587-2596.
41. Shimizu, H., et al., *Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County.* Br J Cancer, 1991. **63**(6): p. 963-6.
42. Cao, Y. and J. Ma, *Body mass index, prostate cancer-specific mortality, and biochemical recurrence: a systematic review and meta-analysis.* Cancer Prev Res (Phila), 2011. **4**(4): p. 486-501.

43. Van Blarigan, E.L., et al., *Fat intake after prostate cancer diagnosis and mortality in the Physicians' Health Study.* Cancer Causes Control, 2015. **26**(8): p. 1117-26.
44. Labbe, D.P., et al., *High-fat diet fuels prostate cancer progression by rewiring the metabolome and amplifying the MYC program.* Nat Commun, 2019. **10**(1): p. 4358.
45. Labbe, D.P., et al., *Role of diet in prostate cancer: the epigenetic link.* Oncogene, 2015. **34**(36): p. 4683-91.
46. Pernar, C.H., et al., *The Epidemiology of Prostate Cancer.* Cold Spring Harb Perspect Med, 2018. **8**(12).
47. Kotake-Nara, E., et al., *Carotenoids affect proliferation of human prostate cancer cells.* J Nutr, 2001. **131**(12): p. 3303-6.
48. Obermuller-Jevic, U.C., et al., *Lycopene inhibits the growth of normal human prostate epithelial cells in vitro.* J Nutr, 2003. **133**(11): p. 3356-60.
49. Beaver, L.M., et al., *Broccoli Sprouts Delay Prostate Cancer Formation and Decrease Prostate Cancer Severity with a Concurrent Decrease in HDAC3 Protein Expression in Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) Mice.* Curr Dev Nutr, 2018. **2**(3): p. nzy002.
50. Catalona, W.J., et al., *Measurement of prostate-specific antigen in serum as a screening test for prostate cancer.* N Engl J Med, 1991. **324**(17): p. 1156-61.
51. Barakzai, M.A., *Prostatic Adenocarcinoma: A Grading from Gleason to the New Grade-Group System: A Historical and Critical Review.* Asian Pac J Cancer Prev, 2019. **20**(3): p. 661-666.
52. Klotz, L., et al., *Long-term follow-up of a large active surveillance cohort of patients with prostate cancer.* J Clin Oncol, 2015. **33**(3): p. 272-7.
53. Welty, C.J., et al., *Extended followup and risk factors for disease reclassification in a large active surveillance cohort for localized prostate cancer.* J Urol, 2015. **193**(3): p. 807-11.
54. Barocas, D.A., et al., *Association Between Radiation Therapy, Surgery, or Observation for Localized Prostate Cancer and Patient-Reported Outcomes After 3 Years.* JAMA, 2017. **317**(11): p. 1126-1140.
55. Chen, R.C., et al., *Association Between Choice of Radical Prostatectomy, External Beam Radiotherapy, Brachytherapy, or Active Surveillance and Patient-Reported Quality of Life Among Men With Localized Prostate Cancer.* JAMA, 2017. **317**(11): p. 1141-1150.
56. Greco, C., et al., *The evolving role of external beam radiotherapy in localized prostate cancer.* Semin Oncol, 2019. **46**(3): p. 246-253.
57. Kamran, S.C. and A.V. D'Amico, *Radiation Therapy for Prostate Cancer.* Hematol Oncol Clin North Am, 2020. **34**(1): p. 45-69.
58. Huggins, C. and C.V. Hodges, *Studies on prostatic cancer. I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate.* CA Cancer J Clin, 1972. **22**(4): p. 232-40.
59. Harris, W.P., et al., *Androgen deprivation therapy: progress in understanding mechanisms of resistance and optimizing androgen depletion.* Nat Clin Pract Urol, 2009. **6**(2): p. 76-85.
60. Saylor, P.J. and M.R. Smith, *Metabolic complications of androgen deprivation therapy for prostate cancer.* J Urol, 2013. **189**(1 Suppl): p. S34-42; discussion S43-4.

61. Poulsen, M.H., et al., *Osteoporosis and prostate cancer; a 24-month prospective observational study during androgen deprivation therapy.* Scand J Urol, 2019. **53**(1): p. 34-39.
62. Yin, L. and Q. Hu, *CYP17 inhibitors--abiraterone, C17,20-lyase inhibitors and multi-targeting agents.* Nat Rev Urol, 2014. **11**(1): p. 32-42.
63. Quinn, D.I., et al., *The evolution of chemotherapy for the treatment of prostate cancer.* Ann Oncol, 2017. **28**(11): p. 2658-2669.
64. Botrel, T.E., et al., *Efficacy and Safety of Combined Androgen Deprivation Therapy (ADT) and Docetaxel Compared with ADT Alone for Metastatic Hormone-Naive Prostate Cancer: A Systematic Review and Meta-Analysis.* PLoS One, 2016. **11**(6): p. e0157660.
65. Chen, J., et al., *Comparison of Current Systemic Combination Therapies for Metastatic Hormone-Sensitive Prostate Cancer and Selection of Candidates for Optimal Treatment: A Systematic Review and Bayesian Network Meta-Analysis.* Front Oncol, 2020. **10**: p. 519388.
66. Kantoff, P.W., et al., *Sipuleucel-T immunotherapy for castration-resistant prostate cancer.* N Engl J Med, 2010. **363**(5): p. 411-22.
67. Grewal, K., K. Grewal, and I.A. Tabbara, *PARP Inhibitors in Prostate Cancer.* Anticancer Res, 2021. **41**(2): p. 551-556.
68. Rahbar, K., et al., *Response and Tolerability of a Single Dose of 177Lu-PSMA-617 in Patients with Metastatic Castration-Resistant Prostate Cancer: A Multicenter Retrospective Analysis.* J Nucl Med, 2016. **57**(9): p. 1334-8.
69. Wang, G., et al., *Genetics and biology of prostate cancer.* Genes Dev, 2018. **32**(17-18): p. 1105-1140.
70. Le, A., S. Udupa, and C. Zhang, *The Metabolic Interplay between Cancer and Other Diseases.* Trends Cancer, 2019. **5**(12): p. 809-821.
71. Klil-Drori, A.J., L. Azoulay, and M.N. Pollak, *Cancer, obesity, diabetes, and antidiabetic drugs: is the fog clearing?* Nat Rev Clin Oncol, 2017. **14**(2): p. 85-99.
72. Pollak, M., *The insulin and insulin-like growth factor receptor family in neoplasia: an update.* Nat Rev Cancer, 2012. **12**(3): p. 159-69.
73. Pothiwala, P., S.K. Jain, and S. Yaturu, *Metabolic syndrome and cancer.* Metab Syndr Relat Disord, 2009. **7**(4): p. 279-88.
74. Bansal, D., et al., *Type 2 diabetes and risk of prostate cancer: a meta-analysis of observational studies.* Prostate Cancer Prostatic Dis, 2013. **16**(2): p. 151-8, S1.
75. Foretz, M., et al., *Metformin: from mechanisms of action to therapies.* Cell Metab, 2014. **20**(6): p. 953-66.
76. Zaidi, S., et al., *The anticancer potential of metformin on prostate cancer.* Prostate Cancer Prostatic Dis, 2019. **22**(3): p. 351-361.
77. Lee, J., E. Giovannucci, and J.Y. Jeon, *Diabetes and mortality in patients with prostate cancer: a meta-analysis.* Springerplus, 2016. **5**(1): p. 1548.
78. Eidelman, E., et al., *The Metabolic Phenotype of Prostate Cancer.* Frontiers in oncology, 2017. **7**: p. 131-131.
79. Ahmad, S., et al., *Do all roads lead to the Rome? The glycation perspective!* Semin Cancer Biol, 2018. **49**: p. 9-19.
80. Zeng, C., et al., *Clinical/Translational Aspects of Advanced Glycation End-Products.* Trends Endocrinol Metab, 2019. **30**(12): p. 959-973.

81. Rahbar, S., *The discovery of glycated hemoglobin: a major event in the study of nonenzymatic chemistry in biological systems.* Ann N Y Acad Sci, 2005. **1043**: p. 9-19.
82. Beltran Del Rio, M., et al., *Glycated Hemoglobin, Plasma Glucose, and Erythrocyte Aging.* J Diabetes Sci Technol, 2016. **10**(6): p. 1303-1307.
83. Furber, J.D., *Extracellular glycation crosslinks: prospects for removal.* Rejuvenation Res, 2006. **9**(2): p. 274-8.
84. Snedeker, J.G. and A. Gautieri, *The role of collagen crosslinks in ageing and diabetes - the good, the bad, and the ugly.* Muscles Ligaments Tendons J, 2014. **4**(3): p. 303-8.
85. Lee, A.T. and A. Cerami, *Role of glycation in aging.* Ann N Y Acad Sci, 1992. **663**: p. 63-70.
86. Tamae, D., et al., *Mutagenesis and repair induced by the DNA advanced glycation end product N2-1-(carboxyethyl)-2'-deoxyguanosine in human cells.* Biochemistry, 2011. **50**(12): p. 2321-9.
87. Anguizola, J., et al., *Review: Glycation of human serum albumin.* Clin Chim Acta, 2013. **425**: p. 64-76.
88. Rabbani, G. and S.N. Ahn, *Structure, enzymatic activities, glycation and therapeutic potential of human serum albumin: A natural cargo.* Int J Biol Macromol, 2019. **123**: p. 979-990.
89. Kouzuma, T., et al., *An enzymatic method for the measurement of glycated albumin in biological samples.* Clin Chim Acta, 2002. **324**(1-2): p. 61-71.
90. Mastrocola, R., et al., *Accumulation of advanced glycation end-products and activation of the SCAP/SREBP Lipogenetic pathway occur in diet-induced obese mouse skeletal muscle.* PLoS One, 2015. **10**(3): p. e0119587.
91. Soboleva, A., et al., *Probing Protein Glycation by Chromatography and Mass Spectrometry: Analysis of Glycation Adducts.* Int J Mol Sci, 2017. **18**(12).
92. Alsamad, F., et al., *In depth investigation of collagen non-enzymatic glycation by Raman spectroscopy.* Spectrochim Acta A Mol Biomol Spectrosc, 2021. **251**: p. 119382.
93. Otero de Joshi, V., et al., *Assessment of nonenzymatic glycation in protein by FTIR spectroscopy*. BiOS '99 International Biomedical Optics Symposium. Vol. 3608. 1999: SPIE.
94. Li, Y., et al., *Advanced Glycation End Products Inhibit the Proliferation of Human Umbilical Vein Endothelial Cells by Inhibiting Cathepsin D.* Int J Mol Sci, 2017. **18**(2).
95. Patel, S.H., et al., *Advanced Glycation End-Products Suppress Mitochondrial Function and Proliferative Capacity of Achilles Tendon-Derived Fibroblasts.* Sci Rep, 2019. **9**(1): p. 12614.
96. Wei, Y., et al., *Rapid glycation with D-ribose induces globular amyloid-like aggregations of BSA with high cytotoxicity to SH-SY5Y cells.* BMC Cell Biol, 2009. **10**: p. 10.
97. Hou, X., et al., *Advanced glycation endproducts trigger autophagy in cadiomyocyte via RAGE/PI3K/AKT/mTOR pathway.* Cardiovasc Diabetol, 2014. **13**: p. 78.
98. Sharaf, H., et al., *Advanced glycation endproducts increase proliferation, migration and invasion of the breast cancer cell line MDA-MB-231.* Biochim Biophys Acta, 2015. **1852**(3): p. 429-41.
99. Matou-Nasri, S., et al., *Biological impact of advanced glycation endproducts on estrogen receptor-positive MCF-7 breast cancer cells.* Biochim Biophys Acta Mol Basis Dis, 2017. **1863**(11): p. 2808-2820.

100. Peterson, L.L., et al., *Dietary advanced glycation end products and the risk of postmenopausal breast cancer in the National Institutes of Health-AARP Diet and Health Study.* Cancer, 2020. **126**(11): p. 2648-2657.
101. Uribarri, J., et al., *Advanced glycation end products in foods and a practical guide to their reduction in the diet.* J Am Diet Assoc, 2010. **110**(6): p. 911-16 e12.
102. Deng, R., et al., *Glucose-derived AGEs promote migration and invasion of colorectal cancer by up-regulating Sp1 expression.* Biochim Biophys Acta Gen Subj, 2017. **1861**(5 Pt A): p. 1065-1074.
103. Katz, J., et al., *Advanced glycation endproducts (AGEs) in saliva of patients with multiple myeloma - a pilot study.* Leuk Lymphoma, 2017. **58**(12): p. 2934-2938.
104. Bao, J.M., et al., *AGE/RAGE/Akt pathway contributes to prostate cancer cell proliferation by promoting Rb phosphorylation and degradation.* Am J Cancer Res, 2015. **5**(5): p. 1741-50.
105. Chen, Y. and T.L. Guo, *Dietary Early Glycation Products Promote the Growth of Prostate Tumors More than Advanced Glycation End-Products through Modulation of Macrophage Polarization.* Mol Nutr Food Res, 2019. **63**(4): p. e1800885.
106. Bonnans, C., J. Chou, and Z. Werb, *Remodelling the extracellular matrix in development and disease.* Nat Rev Mol Cell Biol, 2014. **15**(12): p. 786-801.
107. Humphrey, J.D., E.R. Dufresne, and M.A. Schwartz, *Mechanotransduction and extracellular matrix homeostasis.* Nat Rev Mol Cell Biol, 2014. **15**(12): p. 802-12.
108. Hynes, R.O. and A. Naba, *Overview of the matrisome--an inventory of extracellular matrix constituents and functions.* Cold Spring Harb Perspect Biol, 2012. **4**(1): p. a004903.
109. Wei, M. and P.G. Wang, *Desialylation in physiological and pathological processes: New target for diagnostic and therapeutic development.* Prog Mol Biol Transl Sci, 2019. **162**: p. 25-57.
110. Frantz, C., K.M. Stewart, and V.M. Weaver, *The extracellular matrix at a glance.* J Cell Sci, 2010. **123**(Pt 24): p. 4195-200.
111. Zhen, G. and X. Cao, *Targeting TGFbeta signaling in subchondral bone and articular cartilage homeostasis.* Trends Pharmacol Sci, 2014. **35**(5): p. 227-36.
112. Alberts, B., *Molecular biology of the cell. [Hauptbd.], [Hauptbd.]*. 2015, New York, NY: Garland Science.
113. Sorushanova, A., et al., *The Collagen Suprafamily: From Biosynthesis to Advanced Biomaterial Development.* Adv Mater, 2019. **31**(1): p. e1801651.
114. Verzijl, N., et al., *Effect of collagen turnover on the accumulation of advanced glycation end products.* J Biol Chem, 2000. **275**(50): p. 39027-31.
115. Dandia, H., K. Makkad, and P. Tayalia, *Glycated collagen - a 3D matrix system to study pathological cell behavior.* Biomater Sci, 2019. **7**(8): p. 3480-3488.
116. Bergamaschi, A., et al., *Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome.* J Pathol, 2008. **214**(3): p. 357-67.
117. Levental, K.R., et al., *Matrix crosslinking forces tumor progression by enhancing integrin signaling.* Cell, 2009. **139**(5): p. 891-906.
118. Pickup, M.W., J.K. Mouw, and V.M. Weaver, *The extracellular matrix modulates the hallmarks of cancer.* EMBO Rep, 2014. **15**(12): p. 1243-53.

119. Schwartz, M.A. and R.K. Assoian, *Integrins and cell proliferation: regulation of cyclin-dependent kinases via cytoplasmic signaling pathways.* J Cell Sci, 2001. **114**(Pt 14): p. 2553-60.
120. Keely, P.J., *Mechanisms by which the extracellular matrix and integrin signaling act to regulate the switch between tumor suppression and tumor promotion.* J Mammary Gland Biol Neoplasia, 2011. **16**(3): p. 205-19.
121. Ruoslahti, E. and J.C. Reed, *Anchorage dependence, integrins, and apoptosis.* Cell, 1994. **77**(4): p. 477-8.
122. Gilmore, A.P., et al., *Integrin-mediated survival signals regulate the apoptotic function of Bax through its conformation and subcellular localization.* J Cell Biol, 2000. **149**(2): p. 431-46.
123. Bordeleau, F., et al., *Matrix stiffening promotes a tumor vasculature phenotype.* Proc Natl Acad Sci U S A, 2017. **114**(3): p. 492-497.
124. Leight, J.L., et al., *Matrix rigidity regulates a switch between TGF-beta1-induced apoptosis and epithelial-mesenchymal transition.* Mol Biol Cell, 2012. **23**(5): p. 781-91.
125. Filipe, E.C., J.L. Chitty, and T.R. Cox, *Charting the unexplored extracellular matrix in cancer.* International journal of experimental pathology, 2018. **99**(2): p. 58-76.
126. Neeper, M., et al., *Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins.* J Biol Chem, 1992. **267**(21): p. 14998-5004.
127. Schmidt, A.M., et al., *Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface.* J Biol Chem, 1992. **267**(21): p. 14987-97.
128. Bierhaus, A. and P.P. Nawroth, *Multiple levels of regulation determine the role of the receptor for AGE (RAGE) as common soil in inflammation, immune responses and diabetes mellitus and its complications.* Diabetologia, 2009. **52**(11): p. 2251-63.
129. Taguchi, A., et al., *Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases.* Nature, 2000. **405**(6784): p. 354-60.
130. Fritz, G., *RAGE: a single receptor fits multiple ligands.* Trends Biochem Sci, 2011. **36**(12): p. 625-32.
131. Sparvero, L.J., et al., *RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation.* J Transl Med, 2009. **7**: p. 17.
132. Hudson, B.I., et al., *Interaction of the RAGE cytoplasmic domain with diaphanous-1 is required for ligand-stimulated cellular migration through activation of Rac1 and Cdc42.* J Biol Chem, 2008. **283**(49): p. 34457-68.
133. Yan, S.D., et al., *Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins.* J Biol Chem, 1994. **269**(13): p. 9889-97.
134. Palanissami, G. and S.F.D. Paul, *RAGE and Its Ligands: Molecular Interplay Between Glycation, Inflammation, and Hallmarks of Cancer-a Review.* Horm Cancer, 2018. **9**(5): p. 295-325.
135. Kwak, T., et al., *Targeting of RAGE-ligand signaling impairs breast cancer cell invasion and metastasis.* Oncogene, 2017. **36**(11): p. 1559-1572.
136. Li, J., et al., *Rage induces hepatocellular carcinoma proliferation and sorafenib resistance by modulating autophagy.* Cell Death Dis, 2018. **9**(2): p. 225.

137. Wang, D., et al., *Overexpression of the Receptor for Advanced Glycation Endproducts (RAGE) is associated with poor prognosis in gastric cancer.* PLoS One, 2015. **10**(4): p. e0122697.
138. Kang, R., et al., *The HMGB1/RAGE inflammatory pathway promotes pancreatic tumor growth by regulating mitochondrial bioenergetics.* Oncogene, 2014. **33**(5): p. 567-77.
139. Akiyama, M., et al., *Nuclear factor-kappaB p65 mediates tumor necrosis factor alpha-induced nuclear translocation of telomerase reverse transcriptase protein.* Cancer Res, 2003. **63**(1): p. 18-21.
140. Hanahan, D. and R.A. Weinberg, *The hallmarks of cancer.* Cell, 2000. **100**(1): p. 57-70.
141. Ott, C., et al., *Role of advanced glycation end products in cellular signaling.* Redox Biol, 2014. **2**: p. 411-29.
142. Li, Y.M., et al., *Molecular identity and cellular distribution of advanced glycation endproduct receptors: relationship of p60 to OST-48 and p90 to 80K-H membrane proteins.* Proc Natl Acad Sci U S A, 1996. **93**(20): p. 11047-52.
143. Vlassara, H., et al., *Protection against loss of innate defenses in adulthood by low advanced glycation end products (AGE) intake: role of the antiinflammatory AGE receptor-1.* J Clin Endocrinol Metab, 2009. **94**(11): p. 4483-91.
144. Goh, K.C., et al., *Identification of p90, a prominent tyrosine-phosphorylated protein in fibroblast growth factor-stimulated cells, as 80K-H.* J Biol Chem, 1996. **271**(10): p. 5832-8.
145. Vlassara, H., et al., *Identification of galectin-3 as a high-affinity binding protein for advanced glycation end products (AGE): a new member of the AGE-receptor complex.* Mol Med, 1995. **1**(6): p. 634-46.
146. Fukumori, T., et al., *Endogenous galectin-3 determines the routing of CD95 apoptotic signaling pathways.* Cancer Res, 2004. **64**(10): p. 3376-9.
147. Kobayashi, T., et al., *Transient silencing of galectin-3 expression promotes both in vitro and in vivo drug-induced apoptosis of human pancreatic carcinoma cells.* Clin Exp Metastasis, 2011. **28**(4): p. 367-76.
148. Araki, N., et al., *Macrophage scavenger receptor mediates the endocytic uptake and degradation of advanced glycation end products of the Maillard reaction.* Eur J Biochem, 1995. **230**(2): p. 408-15.
149. Ohgami, N., et al., *CD36, a member of class B scavenger receptor family, is a receptor for advanced glycation end products.* Ann N Y Acad Sci, 2001. **947**: p. 350-5.
150. Ohgami, N., et al., *Scavenger receptor class B type I-mediated reverse cholesterol transport is inhibited by advanced glycation end products.* J Biol Chem, 2001. **276**(16): p. 13348-55.
151. Jono, T., et al., *Lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) serves as an endothelial receptor for advanced glycation end products (AGE).* FEBS Lett, 2002. **511**(1-3): p. 170-4.
152. Shiu, S.W., Y. Wong, and K.C. Tan, *Effect of advanced glycation end products on lectin-like oxidized low density lipoprotein receptor-1 expression in endothelial cells.* J Atheroscler Thromb, 2012. **19**(12): p. 1083-92.
153. Tamura, Y., et al., *FEEL-1 and FEEL-2 are endocytic receptors for advanced glycation end products.* J Biol Chem, 2003. **278**(15): p. 12613-7.
154. Ahmed, H. and D.M. AlSadek, *Galectin-3 as a Potential Target to Prevent Cancer Metastasis.* Clin Med Insights Oncol, 2015. **9**: p. 113-21.

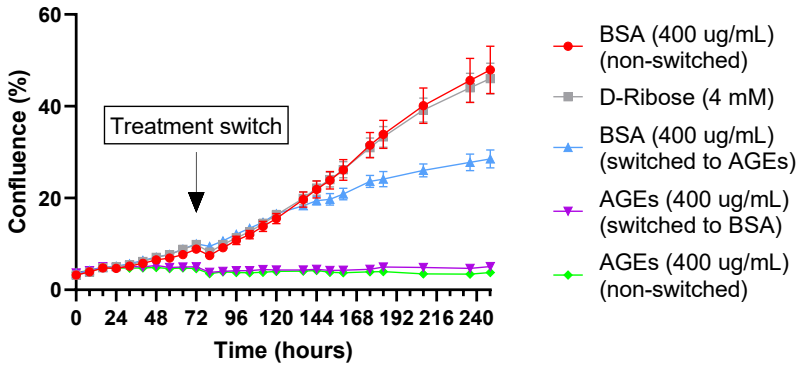
155. Watt, M.J., et al., *Suppressing fatty acid uptake has therapeutic effects in preclinical models of prostate cancer.* Sci Transl Med, 2019. **11**(478).
156. Sacerdote, C. and F. Ricceri, *Epidemiological dimensions of the association between type 2 diabetes and cancer: A review of observational studies.* Diabetes Research and Clinical Practice, 2018. **143**: p. 369-377.
157. Ahmad, S., et al., *Do all roads lead to the Rome? The glycation perspective!* Seminars in Cancer Biology, 2018. **49**: p. 9-19.
158. Watson, P.A., et al., *Context-dependent hormone-refractory progression revealed through characterization of a novel murine prostate cancer cell line.* Cancer Res, 2005. **65**(24): p. 11565-71.
159. Koh, C.M., et al., *MYC and Prostate Cancer.* Genes & cancer, 2010. **1**(6): p. 617-628.
160. Feitelson, M.A., et al., *Sustained proliferation in cancer: Mechanisms and novel therapeutic targets.* Semin Cancer Biol, 2015. **35 Suppl**: p. S25-S54.
161. Wang, X., et al., *Discriminating the Independent Influence of Cell Adhesion and Spreading Area on Stem Cell Fate Determination Using Micropatterned Surfaces.* Scientific Reports, 2016. **6**(1): p. 28708.
162. Paul, C.D., P. Mistriotis, and K. Konstantopoulos, *Cancer cell motility: lessons from migration in confined spaces.* Nature Reviews Cancer, 2017. **17**(2): p. 131-140.
163. Wells, R.G., *The role of matrix stiffness in regulating cell behavior.* Hepatology, 2008. **47**(4): p. 1394-400.
164. He, S. and N.E. Sharpless, *Senescence in Health and Disease.* Cell, 2017. **169**(6): p. 1000-1011.
165. Carpenter, V.J., T. Saleh, and D.A. Gewirtz, *Senolytics for Cancer Therapy: Is All That Glitters Really Gold?* Cancers, 2021. **13**(4): p. 723.
166. Childs, B.G., et al., *Senescent cells: an emerging target for diseases of ageing.* Nature reviews Drug discovery, 2017. **16**(10): p. 718.
167. Saleh, T., et al., *Therapy-induced senescence: An "old" friend becomes the enemy.* Cancers, 2020. **12**(4): p. 822.
168. Mason, B.N. and C.A. Reinhart-King, *Controlling the mechanical properties of three-dimensional matrices via non-enzymatic collagen glycation.* Organogenesis, 2013. **9**(2): p. 70-5.
169. Bansode, S., et al., *Glycation changes molecular organization and charge distribution in type I collagen fibrils.* Sci Rep, 2020. **10**(1): p. 3397.
170. Welsh, K.J., M.S. Kirkman, and D.B. Sacks, *Role of glycated proteins in the diagnosis and management of diabetes: research gaps and future directions.* Diabetes care, 2016. **39**(8): p. 1299-1306.
171. Freitas, P.A.C., L.R. Ehlert, and J.L. Camargo, *Glycated albumin: a potential biomarker in diabetes.* Arch Endocrinol Metab, 2017. **61**(3): p. 296-304.
172. Reynolds, A.N., et al., *Glycated albumin is associated with body mass index in euglycemic adults but is not predictive of postprandial blood glucose response.* J Clin Lab Anal, 2017. **31**(5).
173. Tao, S., et al., *The abnormally high level of uric D-ribose for type-2 diabetics.* Progress in Biochemistry and Biophysics, 2013. **40**(9): p. 816-825.
174. Siddiqui, Z., M. Ishtikhar, and S. Ahmad, *d-Ribose induced glycoxidative insult to hemoglobin protein: An approach to spot its structural perturbations.* International journal of biological macromolecules, 2018. **112**: p. 134-147.

175. Siddiqui, Z., et al., *Prevalence of auto-antibodies against D-ribose-glycated-hemoglobin in diabetes mellitus.* Glycobiology, 2019. **29**(5): p. 409-418.
176. Weiderpass, E., et al., *Reduced risk of prostate cancer among patients with diabetes mellitus.* International journal of cancer, 2002. **102**(3): p. 258-261.
177. Mayne, S.T., M.C. Playdon, and C.L. Rock, *Diet, nutrition, and cancer: past, present and future.* Nature Reviews Clinical Oncology, 2016. **13**(8): p. 504-515.
178. de Polo, A. and D.P. Labbé, *Diet-Dependent Metabolic Regulation of DNA Double-Strand Break Repair in Cancer: More Choices on the Menu.* Cancer Prev Res (Phila), 2021. **14**(4): p. 403-414.
179. Fund, W.C.R. and A.I.f.C. Research, *Food, nutrition, physical activity, and the prevention of cancer: a global perspective*. Vol. 1. 2007: Amer Inst for Cancer Research.
180. Morita, M., et al., *Dietary intervention as a therapeutic for cancer.* Cancer Science, 2021. **112**(2): p. 498-504.
181. Lévesque, S., et al., *Trial watch: dietary interventions for cancer therapy.* Oncoimmunology, 2019. **8**(7): p. 1591878-1591878.
182. Wulaningsih, W., et al., *Serum glucose and fructosamine in relation to risk of cancer.* PLoS One, 2013. **8**(1): p. e54944.

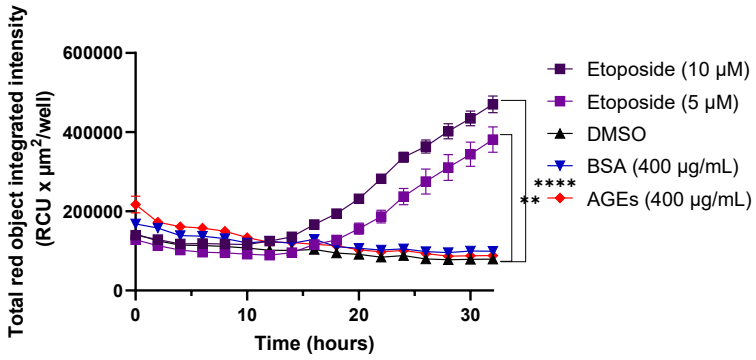
Appendix

Supplementary data for Chapter 2

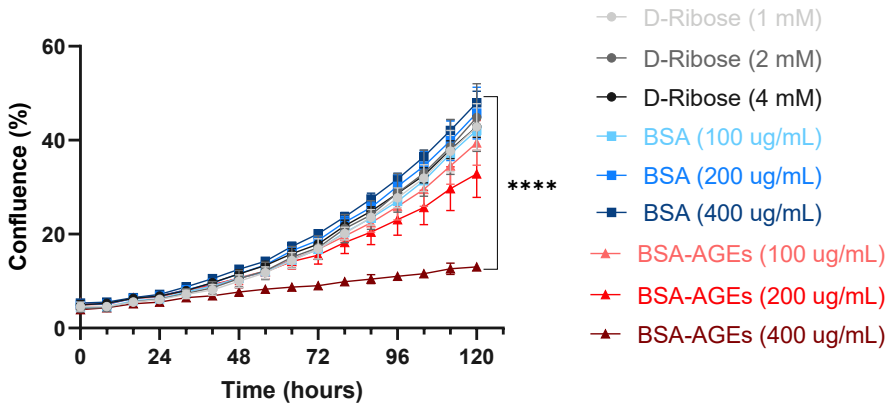
The main objective of this section is to provide the reader with additional information regarding the data presented in **Chapter 2**. Since some of the figures are representative of individual experiments (done with at least three technical replicates), figures from at least one repeat of these experiments are shown here, in order of their appearance in the original manuscript.



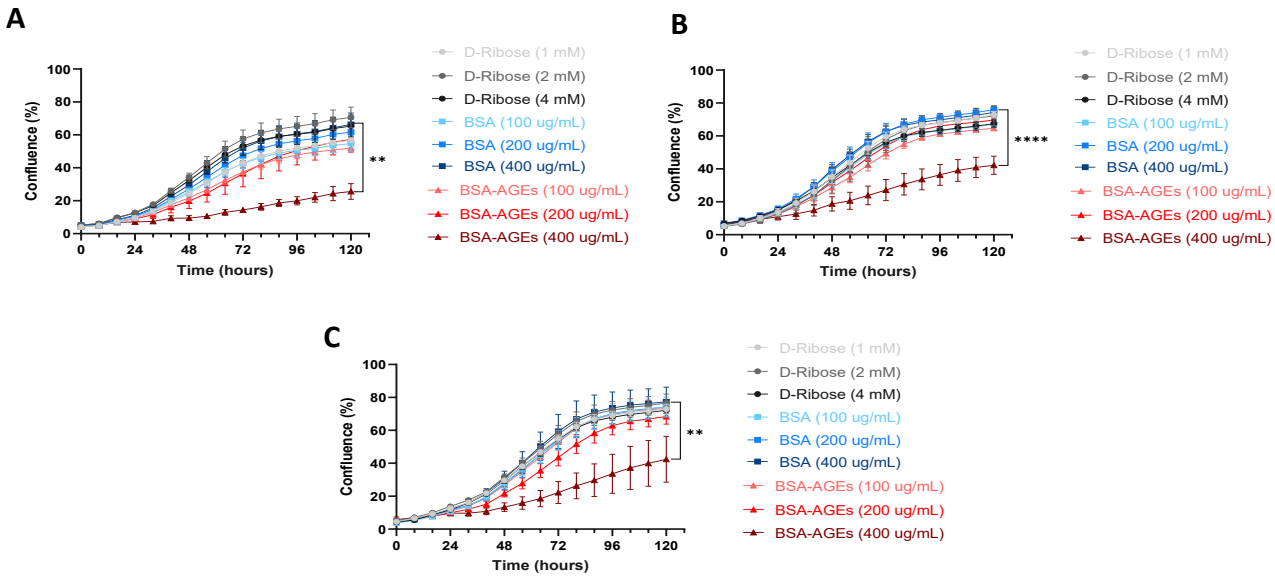
Supplementary Data 1: Another replicate for **Figure 2-4**. Treatment switch does not rescue the proliferation effect mediated by BSA-AGEs in 22Rv1.Data presented as mean ±SEM.



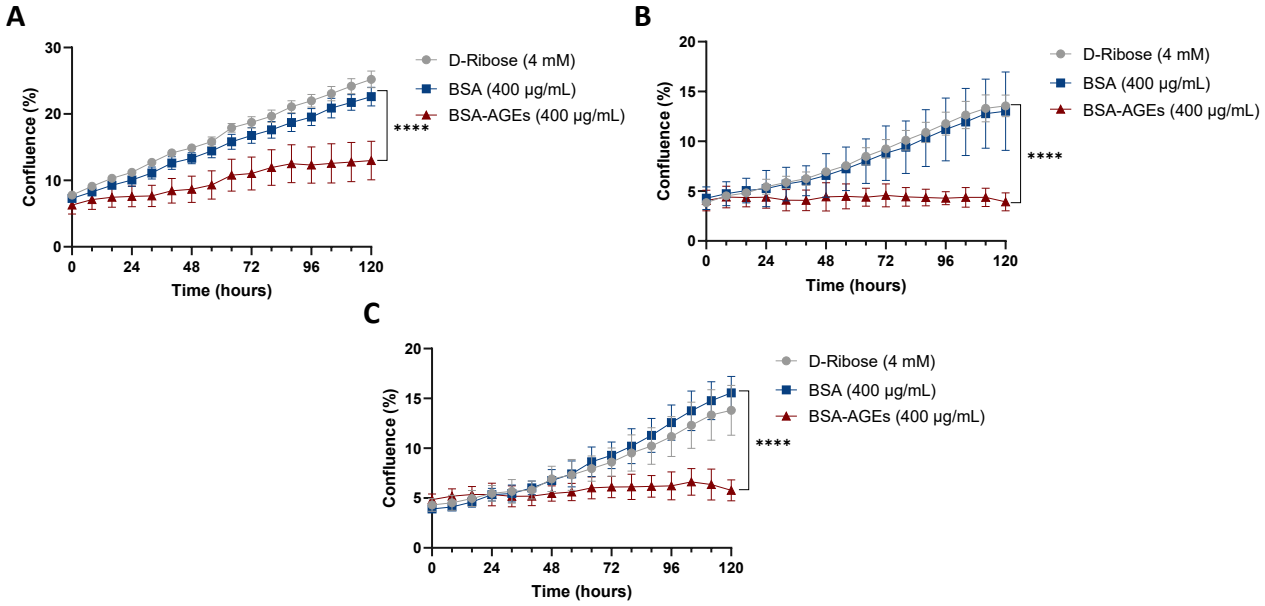
Supplementary Data 2: Another replicate for **Figure 2-5A**. Soluble BSA-AGEs do not induce apoptosis in 22Rv1. Two-way ANOVA. ** $p \leq 0.01$, **** $p \leq 0.0001$.



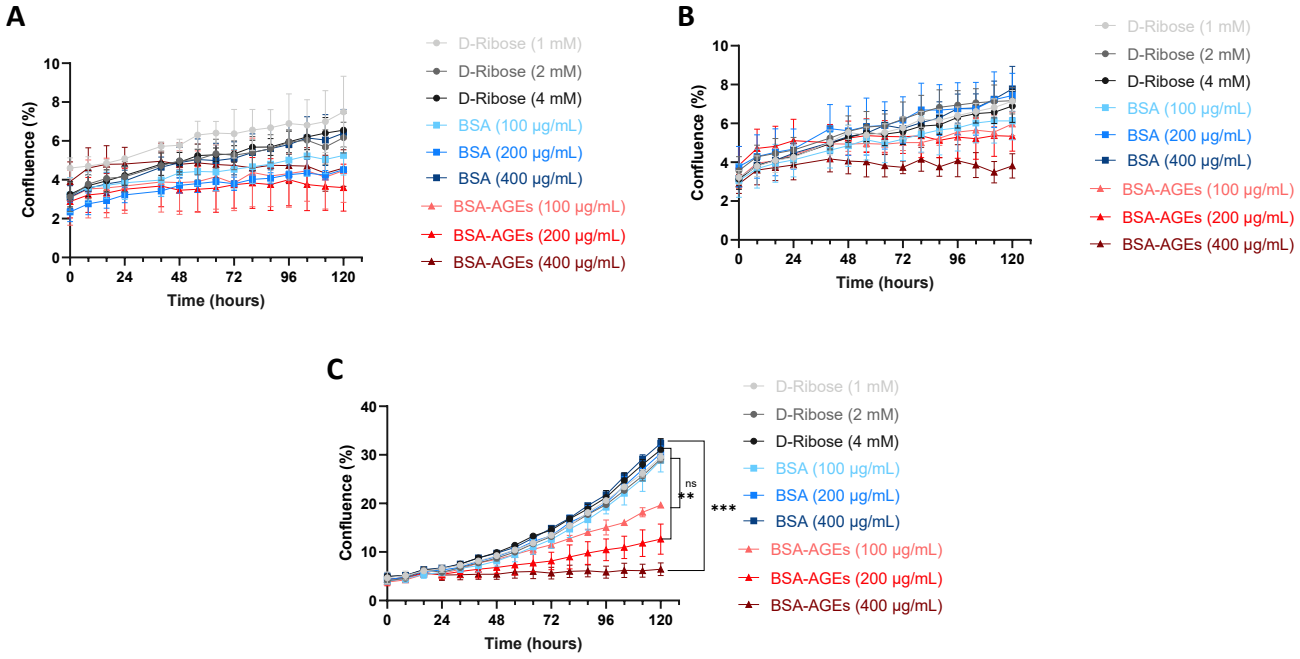
Supplementary Data 3: Another replicate for **Figure S4B**. Soluble BSA-AGEs block proliferation at the optimal dose under 10% FBS. Data represents mean ± SD. Two-way ANOVA. **** $p \leq 0.0001$.



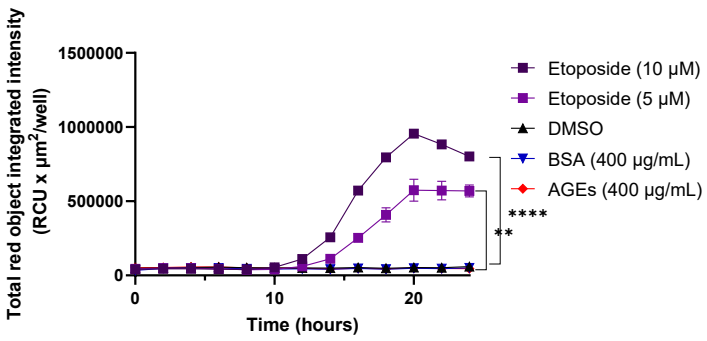
Supplementary Data 4: Another set of replicates for **Figure S5**. Soluble BSA-AGEs block MyC-CaP proliferation across different FBS concentrations: 1% (A), 2% (B) and 10% (C). Data represents mean ± SD. Two-way ANOVA. ** $p \leq 0.01$, **** $p \leq 0.0001$.



Supplementary Data 5: Another set of replicates for **Figure S6**. Soluble BSA-AGEs block LAPC4 proliferation across different FBS concentrations: 1% (A), 2% (B) and 10% (C). Data represents mean ± SD. Two-way ANOVA. **** $p \leq 0.0001$.



Supplementary Data 6: Another set of replicates for **Figure S7**. Effect of soluble BSA-AGEs on 22Rv1 proliferation under 1% (A), 2% (B) and 10% (C) FBS. Data represents mean ± SD. Two-way ANOVA. ns ≥ 0.05, ** p ≤ 0.01, *** p ≤ 0.001.



Supplementary Data 7: Another replicate for **Figure S8A**. Caspase 3-7 cleavage assay in MyC-CaP following BSA-AGEs treatment. Representative experiment shown. Data represents mean ±SEM. Two-way ANOVA. ** $p \leq 0.01$, **** $p \leq 0.0001$.

Appendix

First co-author permission

I, Carole Luthold (postdoctoral fellow, Laval University), grant Tarek Hallal (M.Sc. candidate, McGill University) the permission to use our co-authored manuscript entitled “Soluble advanced glycation endproducts block phenotypes associated with prostate cancer aggressiveness”, as a chapter in his manuscript-based M.Sc. thesis.

Date: 21/06/2021

Signature: