Original Research

Non-enzymatic glycoxidation linked with nutrition enhances the tumorigenic capacity of prostate cancer epithelia through AGE mediated activation of RAGE in cancer associated fibroblasts

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ABSTRACT

The molecular implications of food consumption on cancer etiology are poorly defined. The rate of nutrition associated non-enzymatic glycoxidation, a reaction that occurs between reactive carbonyl groups on linear sugars and nucleophilic amino, lysyl and arginyl groups on fats and proteins, is rapidly increased by food cooking and manufacturing processes. In this study, we assign nutrition-associated glycoxidation with significant oncogenic potential, promoting prostate tumor growth, progression, and metastasis *in vivo*. Advanced glycation end products (AGEs) are the final irreversible product of non-enzymatic glycoxidation. Exogenous treatment of prostate tumor cells with a single AGE peptide replicated glycoxidation induced tumor growth *in vivo*. Mechanistically, receptor for AGE (RAGE) deficiency in the stroma inhibited AGE mediated tumor growth. Functionally, AGE treatment induced RAGE dimerization in activated fibroblasts which sustained and increased the migratory potential of tumor epithelial cells. These data identify a novel nutrition associated pathway that can promote a tissue microenvironment conducive for aggressive tumor growth. Targeted and/or interventional strategies aimed at reducing AGE bioavailability as a consequence of nutrition may be viewed as novel chemoprevention initiatives.

Introduction

The full significance of modern nutrition to cancer etiology is confounded by *a priori* and *a posteriori* dietary studies which often provide conflicting data [1]. Despite this, as many as a third of common cancers are thought to be associated with nutritional intake [2,3]. Molecular models that can reproduce the biological changes associated with multifaceted nutritional habits *in vivo* may help delineate its

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complex synergistic and additive consequences and inform on diet-based strategies for cancer prevention, treatment, and control.

Non-enzymatic glycoxidation is a highly complex reaction resulting from the combined processes of glycation and oxidation. During glycation reactive aldehydes and carbonyls on linear sugars spontaneously bind to free electrophilic amine, lysyl, and arginyl groups on peptides, lipids, and DNA to form a reversible Schiff's base followed by Amadori product [4,5]. Further rearrangement of the carbonyl containing Amadori product can then lead to the formation of irreversible covalent modifications on biological macromolecules called advanced glycation end products (AGEs) (Fig. 1).

During oxidation, the primary glycating sugar on Amadori products can fragment resulting in the production of reactive carbonyl species such as methylglyoxal, glyoxal and deoxyglucosone, which not only increase oxidative stress but are also potent pre-cursors for irreversible AGE formation [4,5].

The rate of non-enzymatic glycoxidation in food is rapidly increased by the high dry heats and pressures applied during cooking (grilling, broiling, and frying) and manufacturing (extrusion, retorting, and irradiation) processes and is dependent upon sugar, fat, and protein macronutrient content [6,7]. Food manufacturers commonly add AGEs, such as N(6)-carboxymethyl lysine (CML), directly to processed foods to improve both taste and appearance [8]. Evidence suggests that only between twenty and fifty percent of ingested CML is excreted from the body, indicating that a considerable level may be metabolized intra-luminally and absorbed into the circulation [9,10]. Due to the common mechanism of formation, *i.e.* glycoxidation, the same AGE adducts associated with nutritional intake reflect those found in normal tissue, and are often increased in diseased tissue [11–13]. A systematic review of 12 randomized controlled diet trials comprising of 293 participants, indicate that high AGE consumption elevates AGE levels in the circulation of normal individuals with highest levels being observed in those with chronic diseases such as type 2 diabetes, cardiovascular disease, and kidney disease [14]. In larger cohort studies nested within both the NIH-AARP Diet and Health Study, and Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, patients in the highest quintile of AGE consumption positively correlated with increased risk of breast and pancreatic cancer [15–17]. Despite clear tumorigenic potential, a cause-and-effect relationship between nutrition associated glycoxidation and cancer etiology has not previously been reported.

In this study, heat driven non-enzymatic glycoxidation was induced in mouse chow with specific macronutrient content. The premise being that when fed to mice it would produce a pro-tumorigenic tissue microenvironment. It is herein reported that nutrition associated non enzymatic glycoxidation has significant oncogenic potential, stimulating prostate tumor growth, progression, and metastasis in both xenograft and spontaneous prostate cancer mouse models. Exogenous treatment of tumor cells with a single AGE peptide successfully replicated the glycoxidation induced tumor growth. The identification of the receptor for AGE (RAGE) expressed in the stroma as the key substrate and effector of AGE tumorigenic function provided key mechanistic insight. AGE mediated activation of RAGE conferred an 'activated' phenotype on fibroblasts to create a pro-tumorigenic microenvironment conducive for tumor growth and progression. The data presented support that increased AGE bioavailability as a consequence of nutrition is pro-tumorigenic. They function to focus additional multidisciplinary studies on the effects of increased AGE bioavailability due to nutritional intake.
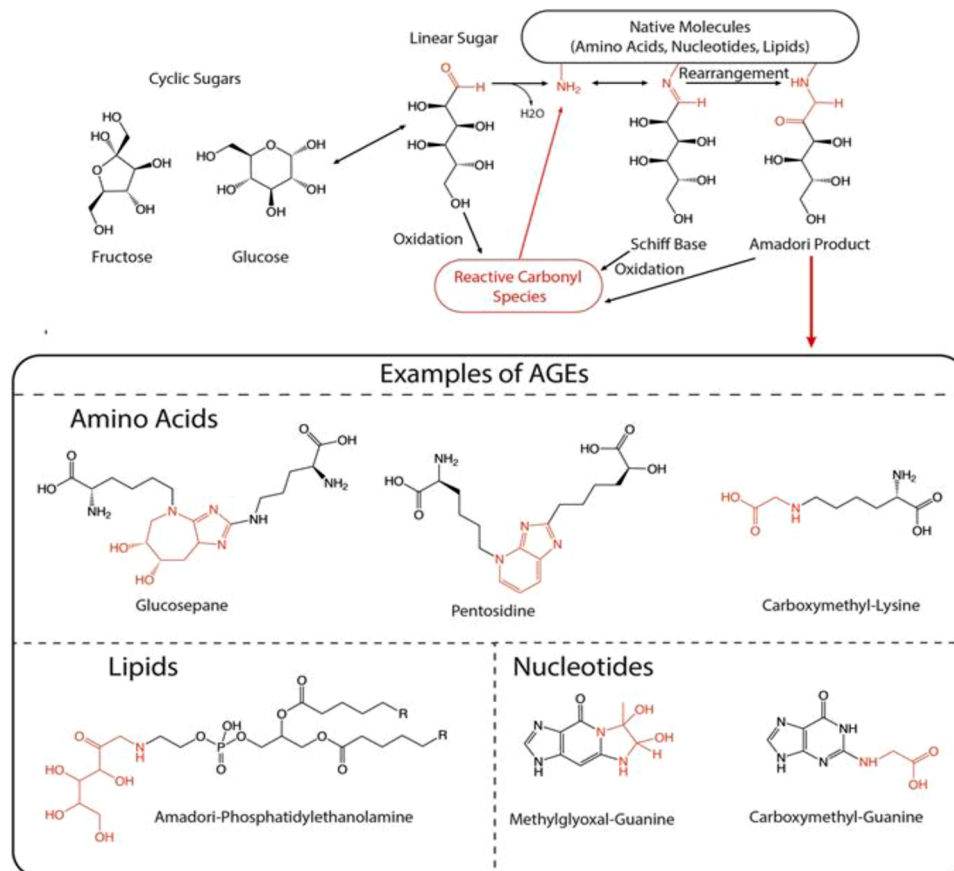

Fig 1. Non-enzymatic glycoxidation schematic. See text for details.

Materials and methods

Animals

Animal care and procedures were approved by the institutional Animal Care and Use Committee at the Medical University of South Carolina (MUSC) and comply with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals. FVB/n and FVB-Tg(C3-1-Tag)cJeg/JegJ (C3-TAg) mice were purchased from Jackson Labs (stock #001800 & #013591) [18]. C57B6 RAGE -/- (Ager[tm1.1Arnd]) [19] mice were a kind gift of Dr. B Arnold (German Cancer Research Center, Heidelberg, Germany) and were backcrossed onto the FVB/n background through 8 generations (FVB-RAGE-/-).

Experimental mouse chows

The experimental diets consisted of: **(1)** Regular: LabDiet 5V75 - 13% k/cal fat, 63% starch; **(2)** Regular control: 13% k/cal fat, 63% starch but this time autoclaved at 120 °C for 15 min [20]; **(3)** High Sugar: Testdiet DIO 58G7 - 12% k/cal fat, 69% glucose; and **(4)** High glycoxidation: TestDiet DIO 58G7 - 12% k/cal fat, 69% glucose but this time autoclaved at 120 °C for 15 min (Fig. 2).

Cell lines and culture models

MYC-CaP, PC3 and WPMY1 cells were purchased from ATCC (Manassas, VA) and cultured as recommended by the supplier. Mycoplasma-negative cultures were ensured by PCR testing and identity regularly confirmed using short tandem repeat profiling (ATCC). For the generation of primary lines, upon patient consent radical prostatectomy tissue was obtained from the operating room using protocols approved by the MUSC Institutional Review Board. Patient matched fibroblasts and epithelial cell lines were then generated and treated with bovine serum albumin derived AGEs (BSA-AGE) (Millipore Sigma, Burlington, MA) as published [21]. In brief, BSA-AGE is produced by the incubation of glucose with BSA at 37 °C which produces a variety of AGE adducts on the BSA peptide backbone. These same adducts are associated with nutritional intake and reflect those found in normal tissue, and are often increased in diseased tissue. Two-compartment culture models were treated with BSA-AGE (25 µg/ml) for 4 h in either the lower (50,000 fibroblasts) or upper (100,000 epithelia) chambers of 6-well Transwells

Fig 2. Major dietary components in the experimental mouse chows. Four experimental mouse chows were fed to mice, regular (starch as main carbohydrate), regular control (heat treated high starch), high sugar (glucose as the main carbohydrate) and high glycoxidation (heat treated high sugar).

(Fisher Scientific, Waltham, MA). Controls comprized of no AGE treatment and AGE treatment of lower and upper compartments in the absence of fibroblasts. In a subset of experiments, fibroblasts were pre-treated with 10 ηM of the RAGE inhibitor TTP-488 (MedChem, Monmouth Junction, NJ) for 1 h before AGE treatment. Migration was assessed as previously published by this group [22].

Syngeneic dietary xenograft mouse models

FVB-RAGE+/+ and FVB-RAGE-/- mice were weaned at 3 weeks old and experimental diets initiated. At 8 weeks old 5×10^5 MYC-CaP mouse prostate cancer epithelial cells were subcutaneously injected into the left flank in Matrigel (1:1 ratio) and tumor growth assessed biweekly until 12 weeks old. MYC-CaP cells were also pretreated with BSA-AGE (Millipore Sigma) or BSA only vehicle at a concentration of 50 µg/ml for 6 passages before injection. Mouse weights were recorded for longitudinal analysis. Blood was collected at the study endpoint. Endpoint tumors were extracted and either paraffin embedded and/or fresh frozen.

Spontaneous prostate cancer mouse model

C3-TAg male hemizygous offspring were weaned at 3 weeks old, and experimental diets initiated. Mice were sacrificed at 24-weeks to assess low-grade PIN progression and at 40 weeks to assess adenocarcinoma and metastatic progression. Blood was collected at baseline and at the 24- and 40-week time points. Prostates were extracted from mice and paraffin embedded for histological analysis.

Endpoint assessment

Methylglyoxal and AGE content was evaluated using ELISA kits (Cell Biolabs, San Diego, CA) and normalized to total protein content. Immunohistochemistry (IHC) and immunofluorescent (IF) assessment, quantitative PCR, and western blot analysis. Antibody suppliers, PCR primer sequences and UPL probe numbers are detailed in Supp. Tables 1 and 2.

Proximity ligation assay

The DuoLink proximity ligation assay (Sigma, St Louis, MO) was performed as per the manufacturers protocol except that a monoclonal RAGE antibody (Abcam, Cambridge, MA – Ab228861) was used instead of a polyclonal antibody, to ensure that only RAGE:RAGE interactions and not RAGE monomers were detected.

Statistical analysis

The nonparametric Wilcoxon rank sum test was used to determine statistical significance using GraphPad. *P*-values are given for each experiment, in general $P < 0.05$ was considered statistically significant. Error bars represent standard deviation of mean for individual experiments. Associations with tumor progression in animal models were assessed using Fisher's Exacts Test.

Results

Nutrition associated glycoxidation promotes prostate tumor growth

Heat was applied to experimental mouse chow containing sugar as its main carbohydrate source in order to drive non-enzymatic glycoxidation with its fat, and protein content. From weaning, mice were fed either this high glycoxidation diet or one of three specific control diets (Fig. 2). Increased glycoxidation was confirmed in the heat-treated high sugar mouse chow by demonstrating increased methylglyoxal (Fig. 3A) and AGE (Fig. 3B) content when compared to the control diets. After 5 weeks
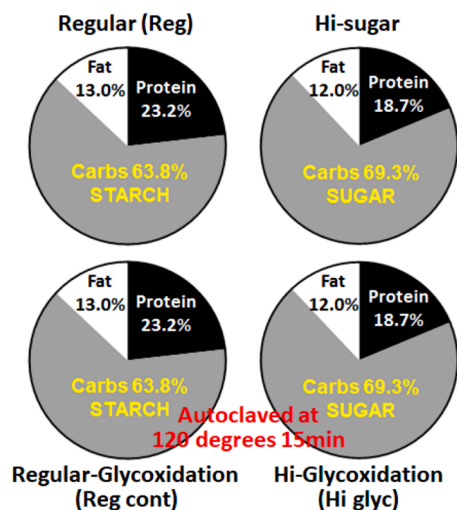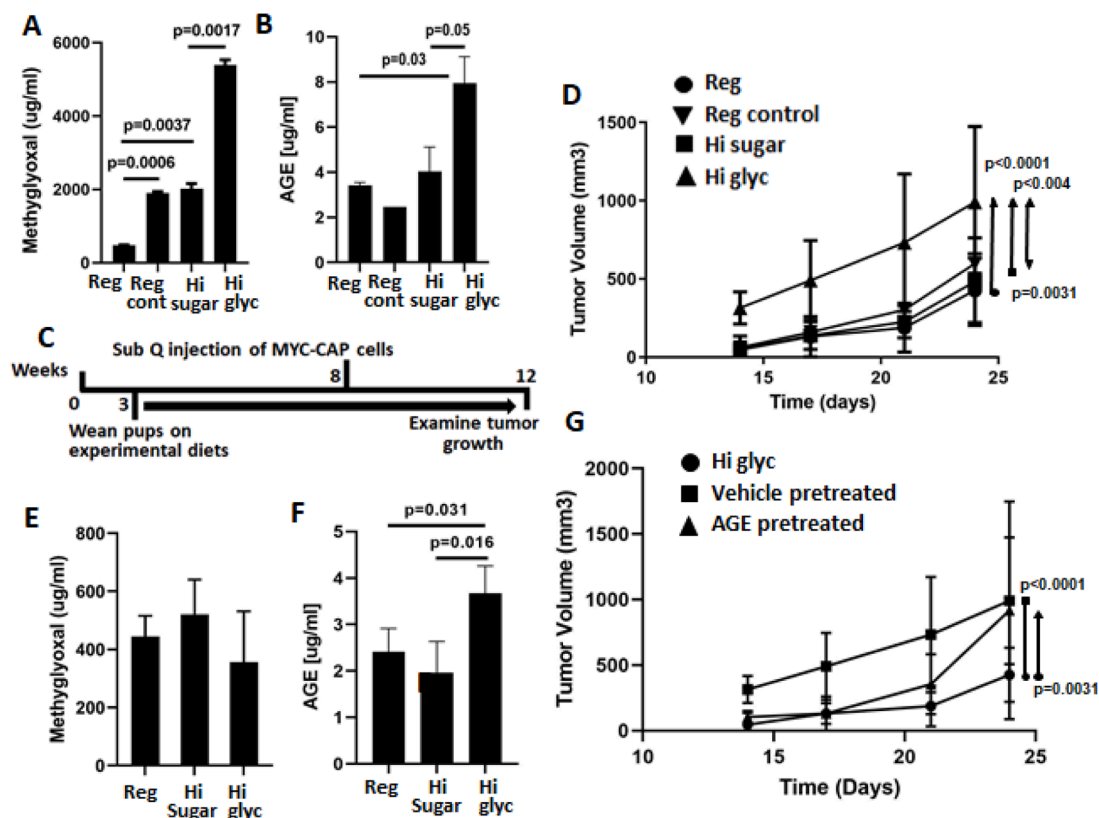
Fig 3. Glycoxidation in food can promote prostate tumor growth. A. Methylglyoxal and B. AGE levels were assessed in the experimental mouse chows by ELISA. C. Schematic outline of the syngeneic xenograft mouse model. D. Tumor growth curves observed in mice fed either regular, regular control, high sugar, or high glycoxidation mouse chow from weaning. E. Methylglyoxal and F. AGE levels were assessed in the circulation of mice fed the experimental mouse chows for 4 weeks by ELISA. G. Tumor growth curves observed in mice fed regular mouse chow injected with MYC-CaP cells pretreated with BSA-AGE peptide (50 µg/ml for 6 passages). Reg=Regular mouse chow; Reg cont=Regular control mouse chow; Hi sugar=high sugar mouse chow; Hi glyc=high glycoxidation mouse chow.

of diet consumption, mouse MYC-CaP prostate cancer cells were injected into the left flank of mice and syngeneic tumor growth assessed biweekly until 12 weeks (Fig. 3C). Consumption of mouse chow high in glycoxidative content significantly accelerated prostate tumor xenograft growth when compared to mice fed the regular chow containing starch as its main carbohydrate source (Fig. 3D). It is noted that the use of heat to induce glycoxidation in food may have confounding effects on other heat labile nutrients. It is also noted that high sugar intake is associated with increased endogenous glycoxidation as well as increased cancer risk [23]. However, when assessed in mice fed either regular mouse chow subjected to the same heat-treatment as the high sugar chow, or unheated high sugar mouse chow, there was no increase in prostate tumor growth over that observed for the regular control (Fig. 3D).

No significant difference in weight gain was observed between mice fed each of the experimental diets (Supp. Fig. 1a).

AGEs are a key pro-tumorigenic dietary factor

While methylglyoxal in food is rapidly degraded in the digestive track, evidence supports that up to 50% of ingested AGEs may be metabolized and absorbed into the circulation [10,24]. Both methylglyoxal and AGE concentrations were assessed in serum from mice fed the regular, high sugar and high glycoxidative mouse chow for a period of 4 weeks. While no significant difference in methylglyoxal levels were observed in the circulation of mice fed the three diets (Fig. 3E), AGE levels were highest in mice fed the mouse chow with high glycoxidative content (Fig. 3F). To directly assess the oncogenic potential of AGEs, MYC-CaP prostate cancer cells were pretreated *in vitro* with BSA control or BSA-AGE peptide (25 µg/ml for 6 passages) before their injection into the flanks of mice fed only regular mouse chow. While tumor growth

was initially delayed, pretreatment with a single AGE peptide increased prostate tumor growth to the same extent as that observed upon the direct consumption of the high glycoxidative mouse chow with high AGE content (Fig. 3G).

Diet intervention failed to reduce glycoxidation induced tumor growth

The effects of nutritional intervention on glycoxidation induced tumor growth were assessed by introducing diet change at the time of xenograft tumor cell injection (Fig. 4A). When mice initially fed mouse chow with high glycoxidative content were then fed regular mouse chow from the time of MYC-CaP cell injection, tumor growth remained at a rate comparable to that observed in mice that were continually fed the high glycoxidative chow (Fig. 4B). In the reverse experiment, no significant increase in tumor growth was observed in mice initially fed regular chow before a change to high glycoxidative chow, although a possible delayed response may have been occurring towards the study endpoint (Fig. 4C).

Nutrition associated glycoxidation drives PIN progression and metastatic potential

The syngeneic xenograft MYC-CaP prostate tumor model lacks the histological characteristics needed to assess the full spectrum of disease progression. Therefore, regular, high sugar, and high glycoxidative mouse chow was fed to male FVB-Tg(C3-1-Tag)cJeg/JegJ (C3-TAg) hemizygous offspring, and the effects on T-antigen driven prostate tumor progression recorded (Fig. 5A) [18]. As assessed in the dorsolateral lobe at 24-weeks, tumor progression towards high grade prostate intraepithelial neoplasia (PIN) was significantly higher in C3-TAg mice
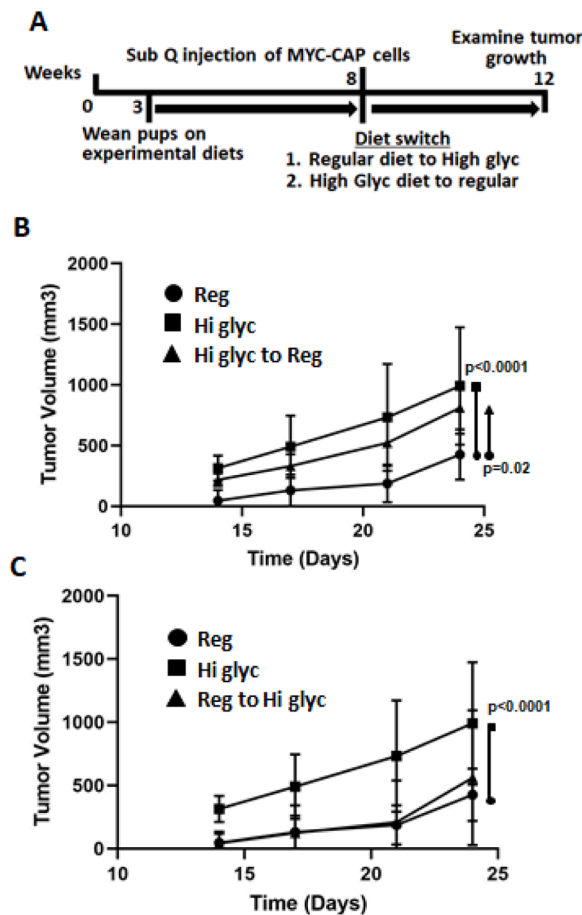
Fig 4. Diet intervention failed to reduce glycoxidation induced tumor growth. A. Schematic outline of the syngeneic xenograft diet intervention mouse model. B. Tumor growth curves observed in mice originally fed the Hi glyc mouse chow followed by Reg mouse chow at the time of MYC-CaP cell injection. C. Tumor growth curves observed in mice originally fed Reg mouse chow followed by Hi glyc mouse chow at the time of MYC-CaP cell injection. Reg=Regular mouse chow; Hi glyc=high glycoxidation mouse chow. Hi glyc to Reg= high glycoxidation mouse chow to Regular switch. Reg to Hi glyc=Regular mouse chow to high glycoxidation switch.

fed the chow with high glycoxidative content when compared to mice fed the regular or high sugar mouse chow (Fig. 5B & Supp. Fig. 1C). No difference in weight gain was observed between mice fed the experimental mouse chows (Supp. Fig. 1b) and no significant differences in tumor progression were observed when assessed in the ventral and anterior lobes of the prostate (data not shown). Specifically, high-grade PIN was seen in 89% of mice fed a high AGE diet at 24 weeks when compared to 38% and 44% of control mice fed a regular diet and high sugar diet respectively (Supp. Fig. 1D). The percentage of high-grade lesions in our control mice were similar to that observed in the original C3-TAg paper [25]. Similarly, at 40 weeks, mice fed the high glycoxidation mouse chow had evidence of more adenocarcinoma (Fig. 5C), and increased occurrence of micro-metastasis to the lung (Fig. 5D). Specifically, we found carcinoma in 67% of mice fed a high AGE diet when compared to 25% of mice fed a regular diet (Supp. Fig. 1E). AGE levels in prostate tumors positively correlate with Gleason grade [26]. IHC staining of the dorsolateral lobes excised from C3-TAg mice show that higher AGE staining intensity in both the stromal and epithelial compartments of mice fed the high glycoxidative mouse chow positively correlates with increased tumor growth at 24 weeks (Fig. 5E & Supp. Fig. 2A). This remained high in the stromal compartment at 40-weeks (Fig. 5F). At 24-weeks AGE staining intensity was significantly lower in the stromal compartment of mice fed the high sugar mouse

chow (Figs. 5E and 7 Supp. Fig. 2A).

Diet mediated pro-tumorigenic effects are dependent upon stromal RAGE expression

Through ligand activation of its cognate receptor RAGE, AGEs alter tissue function through extracellular matrix remodeling and the increased recruitment of inflammatory associated stromal cells [27,28]. Therefore, the functional significance of RAGE to diet mediated tumor growth was assessed in syngeneic xenograft models using RAGE null mice (Supp. Fig. 2C). When RAGE expressing MYC-CaP tumor epithelial cells were injected into RAGE null mice with no host RAGE expression in tissues, prostate tumor growth was prevented in mice fed both the high glycoxidation and regular mouse chow (Fig. 6A). No tumor growth was observed even when the study endpoint was extended to 70 days. However, when assessed by IHC in the dorsolateral lobe of C3-TAg mice, no significant difference in total RAGE protein was observed between the experimental diets at either 24-weeks (Fig. 6B) or 40-weeks (Fig. 6C). In contrast, using *in vitro* proximity ligation assays, we show that AGE treated human WPMY1 fibroblasts have increased activation of RAGE, as demonstrated by distinct RAGE dimerization compared to vehicle treated control cells (Fig. 6D). Therefore, we performed immunofluorescence staining on excised 24-week C3-TAg prostates for RAGE colocalization with the fibroblast marker alpha smooth muscle actin (αSMA). RAGE expression was increased and clearly co-stained with αSMA in the tissue of mice fed the chow high in glycoxidative content while no detectable co-staining was observed in prostates excised from the regular fed mice (Fig. 6E).

AGE mediated RAGE upregulation promotes the recruitment of CAFs

Malignant transformation is associated with an activated stroma orchestrated by extracellular crosstalk between cancer associated fibroblasts (CAFs) and tumor epithelia [29,30]. CAF presence within the dorsolateral lobes of C3-TAg mice was assigned as immunofluorescent co-staining between vimentin and αSMA at the sites of cancer lesions. Compared to mice fed the regular diet, a significant increase in vimentin and αSMA co-staining was observed around PIN and adenocarcinoma lesions in mice fed the high glycoxidation chow at both 24-weeks and 40 weeks, respectively (Fig. 7A and B). Increased vimentin and αSMA co-staining were also confirmed in MYC-CaP xenograft mice fed the same experimental mouse chows (Supp. Fig. 2D). To add human relevance and to confirm *in vivo* observations, the effects of exogenous AGE treatment (25 μg/ml BSA-AGE for 24 h) on CAF marker expression was assessed in *ex vivo* human primary fibroblasts isolated from Gleason 7 radical prostatectomy tissue. AGE treatment resulted in increased collagen type 1 alpha 1 chain A (COL1A1), fibroblast activation proteins alpha (FAP), and fibroblast specific protein (FSP1) transcript expression compared to vehicle control (Fig. 7C). Importantly, this AGE-mediated increase was inhibited by pre-treatment (10 ηM for 1 h) with the RAGE small molecule inhibitor TTP-488 (Fig. 7C).

Functional assessment of CAF activation was performed in two compartment fibroblast and epithelial co-culture models (Fig. 7D). First, resident prostate fibroblasts isolated from wild type FVB/n mice fed either the high glycoxidation or regular chow for 4-weeks, were cultured *ex vivo* with MYC-CaP tumor epithelial cells. No increases in MYC-CaP cell migration were observed by the addition of AGE to the lower compartment in the absence of fibroblasts (Fig. 7E – epithelia only). The migratory potential of MYC-CaP cells was increased in the presence of primary fibroblasts isolated from mice fed both mouse chows, it was significantly higher upon the consumption of the chow high in glycoxidative content (Fig. 7E - black bars). Additionally, the migratory potential of MYC-CaP cells was stimulated further when the primary fibroblasts that had only been previously exposed to AGEs through diet consumption were exposed to treatment with a single AGE peptide (BSA-AGE 25 μg/ml for 4 h) (Fig. 7E - gray bars). Confirmation of the pro-
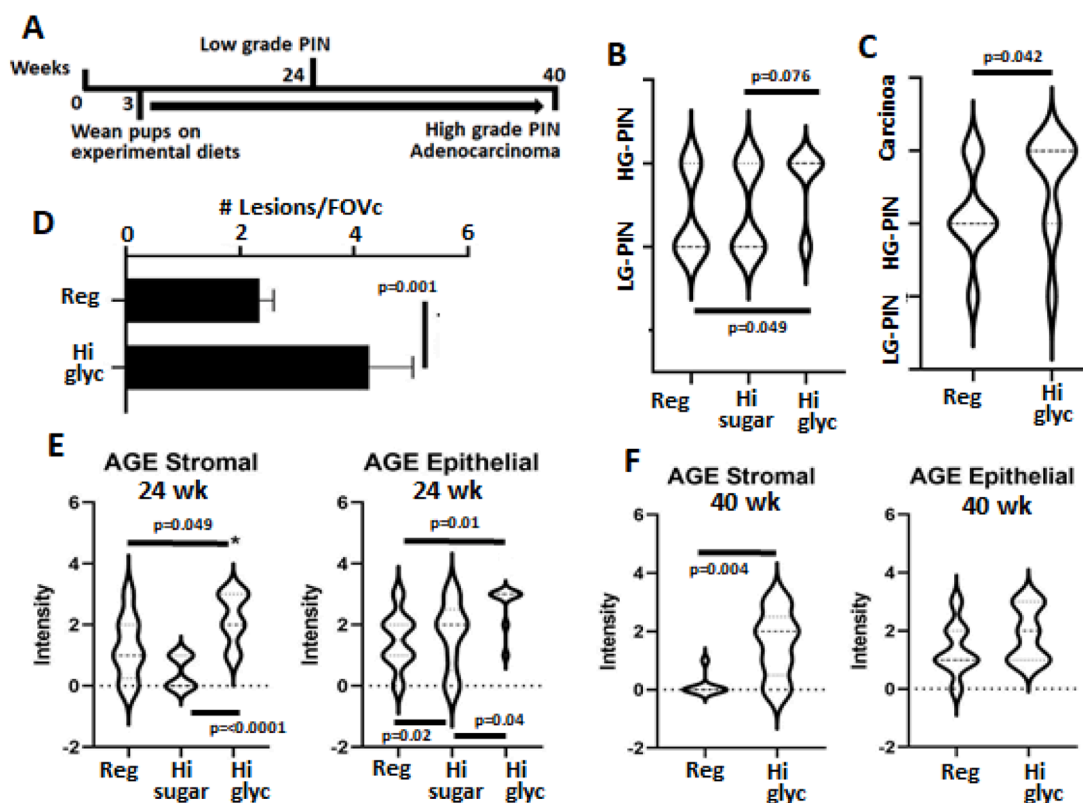
Fig 5. Glycoxidation in food drives prostate tumor progression. A. Schematic outline of the C3-TAg spontaneous prostate cancer model. B. Violin plots of tumor progression observed in C3-TAg mice fed experimental mouse chows at 24 weeks. C. Violin plots of tumor progression observed in C3-TAg mice fed experimental mouse chows at 40 weeks. D. Bar plots of micro-metastasis assessed at 40 weeks in C3-TAg mice fed experimental mouse chow. E. Violin plots of AGE IHC staining intensity in the stroma and epithelial compartments of dorsolateral lobes observed at 24-weeks in C3-TAg mice fed experimental mouse chow. F. Violin plots of AGE IHC staining intensity in the stroma and epithelial compartments of dorsolateral lobes observed at 40-weeks in C3-TAg mice fed experimental mouse chow. Experimental diets are shown in Fig. 2. Reg=Regular mouse chow; Hi sugar=high sugar mouse chow; Hi glyc=high glycoxidation mouse chow.

migratory effects of AGE treated fibroblasts were obtained in co-cultures between human primary fibroblasts and patient matched epithelia isolated from Gleason 7 radical prostatectomy tissue (Fig. 7F) and in the castrate resistant human PC3 tumor epithelial cells when cultured with WPMY1 fibroblasts (Fig. 7G). AGE mediated effects on migratory potential were dependent upon RAGE, as shown by the pre-treatment of fibroblasts with the TTP-488 small molecule RAGE inhibitor (Fig. 7G).

Discussion

A significant caveat to using animal diet models to assess the biological implications of nutrition, is successfully reproducing the complex molecular changes associated with wide variety, combination, and amounts of nutrients being consumed [1]. By using heat induction to drive non-enzymatic glycoxidation in experimental mouse chow, we mimic a consequence of excessive macronutrient consumption, high temperature cooking and processed food manufacturing which involves all three macronutrients. It is again worth noting that the glycoxidation induced peptide-, lipid- and nucleotide-bound AGE adducts found in food are the same as those observed in normal and diseased tissues [11, 12]. In this study, the major difference between the experimental mouse chows was the source of carbohydrate which was starch in the regular and regular control diets, and glucose in the high sugar and high glycoxidation diets (Fig. 2). While the high glycoxidation chow has the same glucose content as the high sugar, high temperature treatment drives the sugar driven formation of reactive carbonyl species and AGEs via the glycoxidation of peptides, lipids and nucleotides [4]. It is recognized that the application of heat to the experimental mouse chow may alter other heat labile potentially pro-tumorigenic nutritional factors. However, key molecular insight from the study supports the high

glycoxidative content as the driver of the observed tumor growth and progression. First, when the same heat treatment was applied to regular mouse chow, it had no effect on prostate tumor growth when fed to mice over that observed for the regular chow (Fig. 3D). Second, AGEs associated with a Western diet arise from two main sources, endogenous formation by glycoxidation events between sugars, fats and proteins in consumed food, and exogenous intake of pre-formed AGEs contained in food before it is consumed. While the consumption of pre-formed AGEs contained in the high glycoxidation chow significantly increased tumor growth and progression, consumption of the high sugar diet did not (Fig. 3D). This indicates that endogenous glycoxidation via sugar consumption, at least at these concentrations, is not a potential nutritional driver of tumor growth. Finally, and critically, the effects on tumor growth (Fig. 3G), fibroblast activation (Fig. 7A–C) and cancer associated processes (Fig. 7E–G) observed in mice fed the high glycoxidation mouse chow were successfully reproduced *in vivo, ex vivo,* and *in vitro* using a single AGE specific peptide.

The pro-tumorigenic consequences of consuming the wide variety and amounts of foods associated with complex dietary patterns, is likely driven by the specific synergistic or additive molecular changes they infer on biological pathways [1]. AGE formation on peptides, nucleic acids and lipids is one of the most prevalent non-enzymatic covalent modifications, it is associated with altered epigenetic profiles and the assembly and stability of nucleosomes, and chromatin architecture [31–33]. The innate plasticity of CAFs supports the premise of a dietary-AGE mediated adaptive response in the tumor microenvironment, which is dependent upon RAGE, and is conducive for aggressive tumor growth. The data reported indicate that nutritionally derived AGEs may exacerbate existing RAGE pro-tumorigenic signals in the stromal compartment. This is supported by the observation that stromal
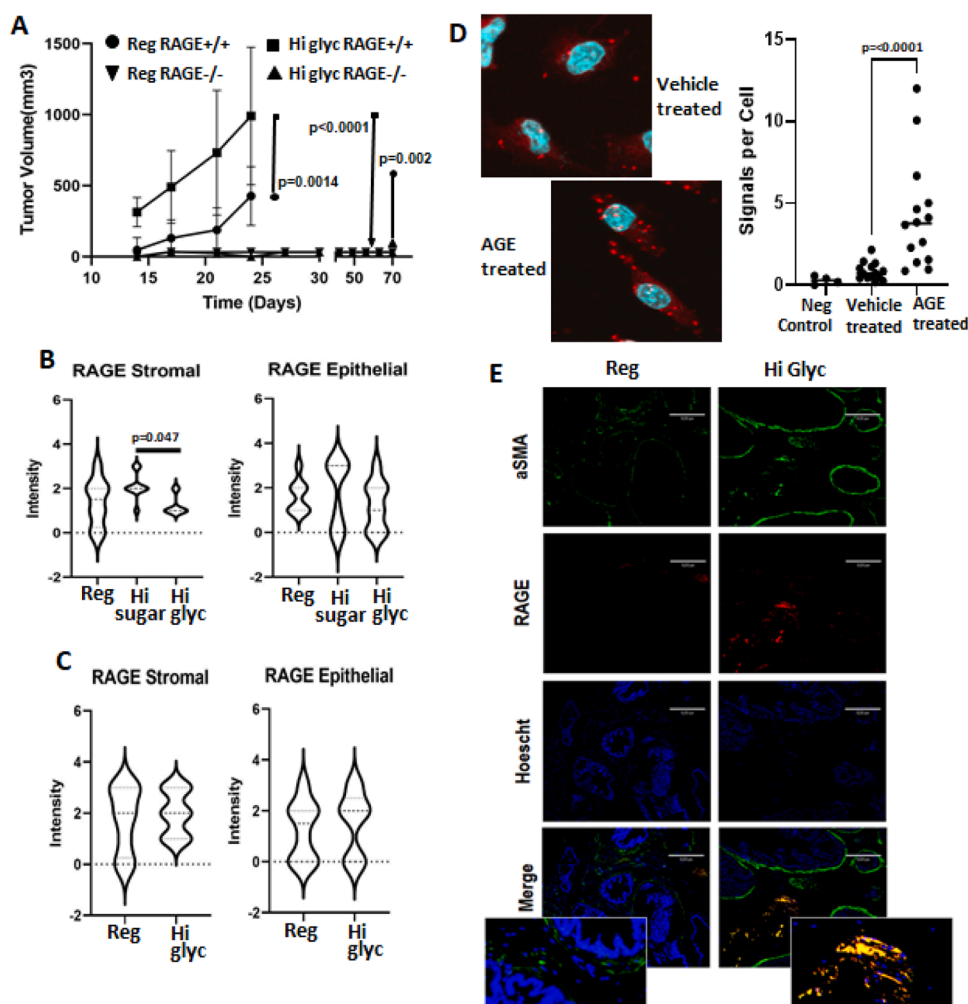
Fig 6. Stromal RAGE expression is a driver of AGE mediated prostate tumor growth. A. Tumor growth curves observed in RAGE+/+ and RAGE-/- MYC-CaP xenograft mice fed experimental mouse chow. B. Violin plots of RAGE IHC staining intensity in the stromal and epithelial compartments of dorsolateral lobes observed at 24-weeks in C3-TAg mice fed experimental mouse chow. C. Violin plots of RAGE IHC staining intensity in the stromal and epithelial compartments of dorsolateral lobes observed at 40-weeks in C3-TAg mice fed experimental mouse chow. D. Representative images and subsequent quantification of RAGE dimerization proximity ligation assays in WPMY1 fibroblasts after treatment with 25 μg/ml BSA-AGE for 4 h. E. Immuno-fluorescence staining of RAGE colocalization with the fibroblast marker αSMA in 24-week dorsolateral PIN lesions excised from mice fed experimental mouse chow. Scale bar = 90 uM. Reg=Regular mouse chow; Hi sugar=high sugar mouse chow; Hi glyc=high glycoxidation mouse chow.

RAGE loss in both regular and high AGE fed mice prevented tumor growth (Fig. 6A). In multiple chronic conditions, AGE-RAGE signaling is associated with persistent inflammatory and oxidative stresses, hormonal and immune dysregulation [34,35], as well as changes to the microbiome [10], epigenome [36], and metabolome [37]. In invasive and non-invasive prostate cancer cells, RAGE is preferentially bound by AGE over other potential ligands such as S100 calcium binding protein B [38]. AGEs were first proposed as a target for prostate cancer therapy over a decade ago. Treatment with a single AGE peptide was shown to stimulate the growth and invasive capacity of castrate resistant prostate cancer cells through activation of RAGE leading to PI3K-AKT mediated degradation of retinoblastoma protein [39]. AGE-dependent modification of the basal lamina has also been shown to induce invasive characteristics in non-transformed prostate epithelial cells [40]. AGE and RAGE levels positively correlate with increased prostate cancer risk and aggressive disease [26,41]. In addition, several aspects of the data indicate that the key pro-tumorigenic changes resulting from chronic AGE consumption are sustained in their effects. Diet intervention failed to reduce tumor growth in mice initially fed a high AGE diet (Fig. 4B) and diet exposed resident prostate fibroblasts retained tumor promoting abilities in *ex vivo* models despite no further AGE exposure once isolated from the mice (Fig. 7E). RAGE function can be self-regulated by splice variants and/or proteolytic cleavage of membrane bound RAGE (sRAGE) that comprise of only the RAGE N-terminal ligand binding domain resulting in competitive ligand sequestration [42,43]. In addition scavenger receptors types I and II, include CD36, CD163 and LOX-1 also sequestor AGE ligand [42]. To further understand the effects of AGEs on the tumor microenvironment additional studies should examine the tumor suppressive and oncogenic roles of AGE targeted receptors.

The release of the 2015–2020 Dietary Guidelines for Americans (www.health.gov) identify the need for holistic approaches in defining the pathogenic contribution of nutrition to non-communicable diseases such as cancer. Controlled interventions, prospective observational and clinical studies, when combined with biological multi-omics and mechanistic studies would inform on data-driven AGE bioavailability and its biological consequences. These data assign AGE consumption as a pro-tumorigenic consequence of modern dietary patterns. They serve to focus additional studies on the oncogenic potential of modern dietary habits known to increase AGE bioavailability. AGE exposure as a consequence of nutrition has potential as a ubiquitous dietary factor to inform on data-driven dietary patterns with which to base cancer prevention guidelines for specific cancer types. Educational, interventional, and pharmacological strategies aimed at reducing AGE bioavailability may be viewed as preventive and/or therapeutic initiatives especially when combined with current cancer treatment regimens.

CRediT authorship contribution statement

Bradley A. Krisanits: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Pamela Woods:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. **Lourdes M. Nogueira:** Formal analysis, Investigation,
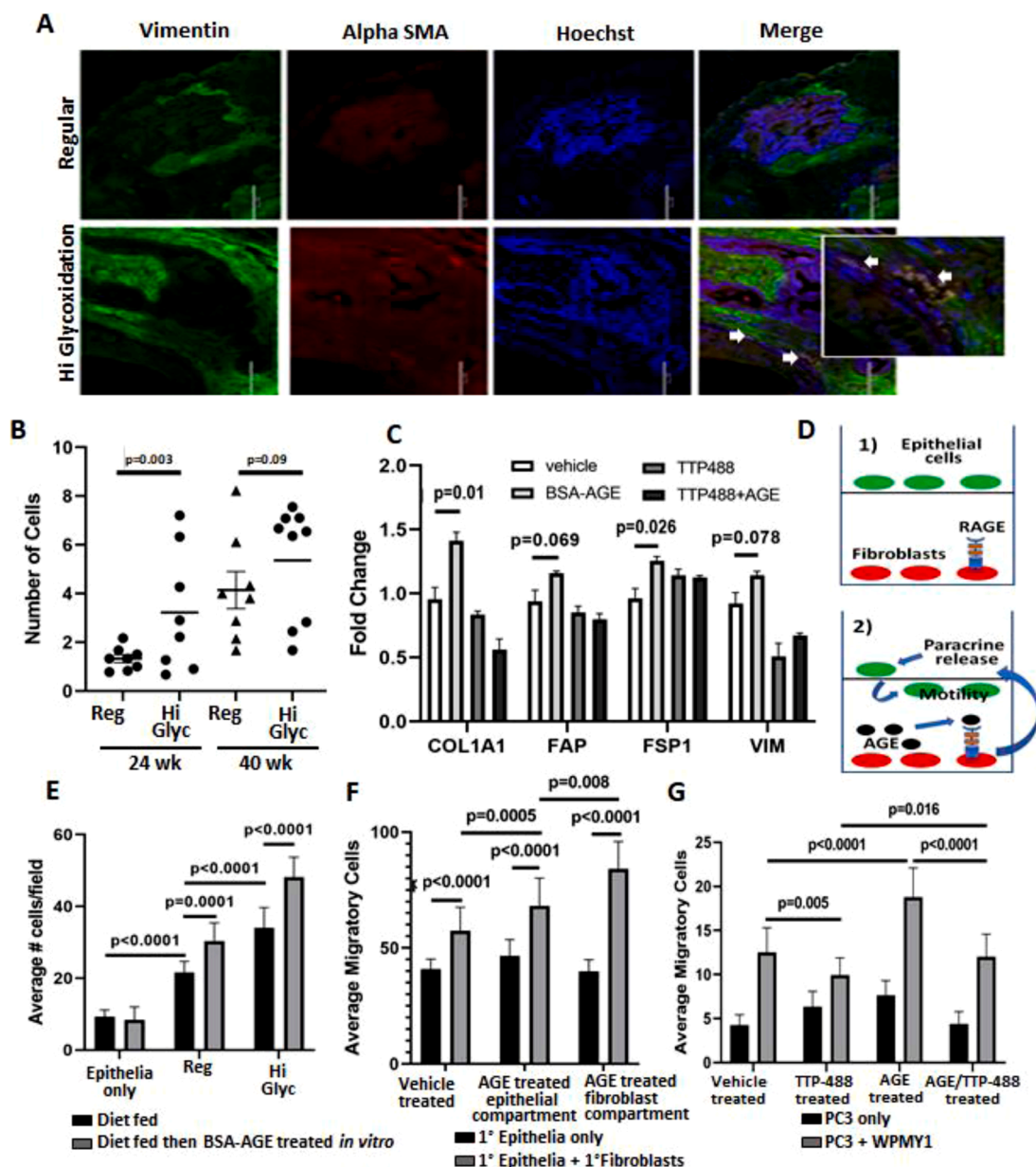
Fig 7. AGE-RAGE signaling increases CAF activation. A. Immuno-fluorescence staining of vimentin colocalization with αSMA in 24-week dorsolateral PIN lesions excised from mice fed experimental mouse chow. Scale bar = 90uM. B. Quantification of vimentin colocalization with αSMA in 24- and 40-week dorsolateral PIN lesions excised from mice fed experimental mouse chow. C. Bar chart of CAF marker expression in WPMY1 fibroblasts treated for 24 h with exogenous AGE (25 μg/ml) in the presence and absence of the RAGE inhibitor TTP-488 (10 ηm). D. Schematic of the two compartment culture model E. Bar charts of the migratory abilities of resident prostate fibroblasts isolated from mice fed a regular and high glycoxidation mouse chow for 4 weeks either untreated (black bars) or treated (gray bars) with BSA-AGE for 4 h. F. Bar chart of the migratory abilities of primary prostate epithelial cells isolated from Gleason 7 radical prostatectomy tissue and cultured with patient matched primary fibroblasts for 4 h in the presence of BSA-AGE (25 μg/ml). Graphs represent data from one of three cell lines derived from Gleason score 7 tissues G. Bar charts of the migratory ability of PC3 human prostate cancer cells when cultured with WPMY1 fibroblasts for 4 h in the presence of BSA-AGE (25 μg/ml) and the RAGE inhibitor TTP-488 (10 ηM).

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Declaration of Competing Interest

DPT and VJF are Co-Founders of the not for profit Anti-A.G.E. Foundation. All other authors declare no potential conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2022.101350.

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