Perturbateurs endocriniens et cancer : Les bisphénols pourraient-ils avoir un impact sur l'initiation et la progression du cancer de la vessie?

Mémoire

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Mémoire de maîtrise

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Sous la direction de :

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Résumé

Les bisphénols sont des composés synthétiques utilisés dans la synthèse des plastiques. Ils ont la capacité de se lier à plusieurs récepteurs cellulaires, dont certains récepteurs hormonaux, ce qui leur confère des propriétés de perturbateurs endocriniens. Certains de ces récepteurs hormonaux sont, entre autres, présents au niveau de l'urothélium de la vessie. De plus en plus d'études ont démontré que l'exposition aux bisphénols est associée à la progression tumorale, surtout pour les cancers hormono-dépendants tels que le cancer de la prostate. La vessie n'est pas considérée comme un tissu hormono-sensible, mais des études ont démontré le rôle des récepteurs hormonaux dans l'initiation et la progression de cancer de la vessie. Étant donné la présence de ces composés dans l'urine chez l'humain, nous avions pour objectif de déterminer si l'exposition chronique aux bisphénols pourrait avoir un impact sur l'initiation et la progression du cancer de la vessie. Ainsi, nous avons exposé des cellules urothéliales saines, des cellules cancéreuses non-invasives et cancéreuses invasives de vessie, des fibroblastes vésicaux et des fibroblastes associés au cancer à des concentrations physiologiques de bisphénols. Nous avons ensuite caractérisé l'impact de cette exposition sur le métabolisme énergétique et l'activité physiologique des cellules. Nous avons observé que les cellules urothéliales et stromales saines présentaient une diminution de ces caractéristiques par rapport aux contrôles, tandis que ces paramètres chez les cellules cancéreuses et les fibroblastes associés au cancer étaient augmentés. Ainsi, l'exposition chronique aux bisphénols semble favoriser la progression des cancers de vessie non-invasifs en cancers invasifs. Ce projet de recherche a permis d'apporter des informations novatrices sur l'impact de perturbateurs endocriniens sur le développement du cancer de la vessie.

Abstract

Bisphenols are synthetic compounds used to produce plastics. They can bind to several cell receptors, including some hormonal receptors, which confers them endocrine-disrupting properties. Some of these hormonal receptors can be found in the urothelium of the bladder. Studies have shown that exposure to bisphenols is associated with tumor progression, especially for hormone-dependent cancers such as prostate cancer. Although the bladder is not recognized as a hormone-sensitive tissue, studies have shown the role of hormonal receptors on bladder cancer initiation and progression. Since these compounds are found in most human urine samples, our objective was to determine whether chronic exposure to bisphenols could have an impact on the initiation and progression of bladder cancer. Therefore, we exposed healthy urothelial cells, non-invasive and invasive bladder cancer cells, bladder fibroblasts, and cancer-associated fibroblasts to physiological concentrations of bisphenols. We then characterized the impact of this exposure on the energy metabolism and physiological activity of the cells. We observed that healthy urothelial and stromal cells exhibited decreased characteristics compared to the controls, while these parameters were increased in cancer cells and cancer-associated fibroblasts. Thus, chronic exposure to bisphenols appears to promote the progression of non-invasive bladder cancers to invasive cancers. This research project provided innovative information on the impact of endocrine disruptors on the development of bladder cancer.

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Liste des abréviations, sigles, acronymes

Abréviations

ADT: *androgen-deprivation therapy* (thérapie de privation d'androgènes)

Akt: *alpha serine-threonine protein kinase* (protéine kinase sérine-thréonine alpha)

AR: *androgen receptor* (récepteur des androgènes)

ARE: *androgen response element* (élément de réponse aux androgènes)

ATF4: *activating transcription factor 4* (activateur de facteur de transcription 4)

ATP: *adenosine triphosphate* (adénosine triphosphate)

BCa: *bladder cancer*

Bax: *Bcl-2–associated X protein* (protéine X associée à Bcl-2)

Bcl-2: *B-cell lymphoma-2* (lymphome à cellules B)

BP: *bisphenol* (bisphénol)

BPA: *bisphenol A* (bisphénol A)

BPS: *bisphenol S* (bisphénol S)

CaV: cancer de la vessie

CAF: *cancer-associated fibroblast* (fibroblaste associé au cancer)

CD: *cluster of differentiation* (classe de différenciation)

CRPC: *castrate-resistant prostate cancer* (cancer de la prostate résistant à la castration)

DDT: *dichlorodiphenyltrichloroethane* (dichlorodiphényltrichloroéthane)

DES: *diethylstilbestrol* (diéthylstilbestrol)

ED: *endocrine disruptor* (perturbateur endocrinien)

EGFR: *epidermal growth factor receptor* (récepteur du facteur de croissance épidermique)

ELK1: *erythroblast transformation specific like-1 protein* (protéine spécifique à la transformation des érythroblastes)

ER: *estrogen receptor* (récepteur des estrogènes)

ERα: *estrogen receptor alpha* (récepteur des estrogènes alpha)

ERβ: *estrogen receptor beta* (récepteur des estrogènes bêta)

ERK: *extracellular signal-regulated kinase* (kinase régulée par signaux extracellulaires)

ERRα: *estrogen-related receptor alpha* (récepteur alpha lié aux estrogènes)

ERRγ: *estrogen-related receptor gamma* (récepteur gamma lié aux estrogènes)

$FADH_2$: *dihydroflavin adenine dinucleotide* (dihydroflavine adénine dinucléotide)

FOXO1: *forkhead box protein O1* (protéine forkhead box O1)

GPER: *G protein-coupled estrogen receptor* (récepteur d'estrogènes couplé aux protéines G)

GTP: guanosine triphosphate

IGF-1R: *insulin-like growth factor-1 receptor* (récepteur du facteur de croissance analogue à l'insuline 1)

IL-6: *interleukin-6* (interleukine-6)

MAPK: *mitogen-activated protein kinase* (protéine kinase activée par un mitogène)

MMP: *matrix metalloproteinase* (métalloprotéinase matricielle)

mTOR: *mechanistic target of rapamycin* (cible mécanistique de la rapamycine)

NAD/NADH: *nicotinamide adenine dinucleotide* (nicotinamide adénine dinucléotide)

p53: *tumour protein 53* (protéine tumorale 53)

PCa: *prostate cancer* (cancer de la prostate)

PI3K: phosphoinositide 3-kinase

PSA: *prostate-specific antigen* (antigène prostatique spécifique)

PSCA: *prostate stem cell antigen* (antigène de cellules souches prostatiques)

RNA: *ribonucleic acid* (acide ribonucléique)

TDS: *testicular dysgenesis syndrome* (syndrome de dysgénésie testiculaire)

TGF-β: *transforming growth factor-β* (facteur de croissance transformant bêta)

TSPAN1: tetraspanin 1

YY1: Yin Yang 1

Remerciements

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Avant-propos

Au cours de mes études à la maîtrise, j'ai eu l'opportunité de participer à la rédaction de plusieurs articles, dont plusieurs à titre de première auteure. Une partie de ces résultats est présentée dans ce rapport final. Les différents articles sont présentés dans les chapitres 1, 2 et 3 de ce mémoire. Les articles des chapitres 1 et 3 ont été révisés par les pairs et publiés. L'article du chapitre 2 a été soumis au journal *Cancers* et est présentement en évaluation. Les différents articles insérés dans ce rapport sont détaillés ci-dessous.

Chapitre 1 : Les effets de perturbateurs endocriniens des bisphénols sur les cancers urologiques

Endocrine-disrupting effects of bisphenols on urological cancers

Pellerin*, E., Caneparo*, C., Chabaud, S., Bolduc, S., & Pelletier, M. (2021). Endocrine-disrupting effects of bisphenols on urological cancers. *Environmental research*, *195*, 110485. https://doi.org/10.1016/j.envres.2020.110485

Journal : Environmental Research

Facteur d'impact (2020) : 6,498

Type d'article : article de revue

Date de soumission : 9 septembre 2020

Date d'acceptation : 11 novembre 2020

Date de publication : 16 novembre 2020

Statut d'auteur : coauteure principale

Rôle dans la préparation de l'article : recherche bibliographique, rédaction du manuscrit, révision finale de l'article

Coauteurs : Christophe Caneparo, Dr Stéphane Chabaud, Dr Martin Pelletier et Dr Stéphane Bolduc

Chapitre 2 : Le bisphénol A et le bisphénol S altèrent le métabolisme énergétique et le comportement des cellules urothéliales saines et cancéreuses de vessie

Bisphenols A and S alter the bioenergetics and behaviours of normal urothelial and bladder cancer cells

Pellerin, È., Pellerin, F. A., Chabaud, S., Pouliot, F., Bolduc, S., & Pelletier, M. (2022). Bisphenols A and S alter the bioenergetics and behaviours of normal urothelial and bladder cancer cells. *Cancers*, *14*(16), 4011. https://doi.org/10.3390/cancers14164011

Journal : Cancers

Facteur d'impact (2022) : 6,886

Type d'article : article original

Date de soumission : 18 juillet 2022

Date d'acceptation : 15 août 2022

Date de publication : 19 août 2022

Statut d'auteur : auteure principale

Rôle dans la préparation de l'article : élaboration et planification des expériences, acquisition, analyse et interprétation des données, rédaction du manuscrit, révision finale de l'article

Coauteurs : Félix-Antoine Pellerin, Dr Stéphane Chabaud, Dr Frédéric Pouliot, Dr Martin Pelletier et Dr Stéphane Bolduc

Chapitre 3 : Le bisphénol A altère le métabolisme énergétique des cellules stromales et pourrait promouvoir la progression du cancer de la vessie

Bisphenol A Alters the Energy Metabolism of Stromal Cells and Could Promote Bladder Cancer Progression

Pellerin, È., Chabaud, S., Pouliot, F., Pelletier, M., & Bolduc, S. (2021). Bisphenol A Alters the Energy Metabolism of Stromal Cells and Could Promote Bladder Cancer Progression. *Cancers*, *13*(21), 5461. https://doi.org/10.3390/cancers13215461

Journal : Cancers

Facteur d'impact (2021) : 6,639

Type d'article : article original

Date de soumission : 1er octobre 2021

Date d'acceptation : 28 octobre 2021

Date de publication : 30 octobre 2021

Statut d'auteur : auteure principale

Rôle dans la préparation de l'article : élaboration et planification des expériences, acquisition, analyse et interprétation des données, rédaction du manuscrit, révision finale de l'article

Coauteurs : Dr Stéphane Chabaud, Dr Frédéric Pouliot, Dr Martin Pelletier et Dr Stéphane Bolduc

Afin d'approfondir davantage mes connaissances dans le domaine du génie tissulaire, j'ai eu l'occasion de participer à la rédaction de deux autres articles scientifiques plus ou moins en lien avec le sujet de ma maîtrise. Le premier article scientifique, publié en novembre 2021 dans le journal *Bioengineering*, m'a permis de bonifier mes connaissances sur la culture cellulaire (**Annexe A.I**), alors que le deuxième article scientifique, publié en mai 2022 dans le journal *Oncology Letters*, m'a permis d'acquérir des connaissances supplémentaires sur l'hypoxie associée au cancer (**Annexe A.II**). Afin de mettre en valeur ces travaux, les informations spécifiques et les résumés relatifs à ces articles ont été ajoutés en annexe de ce mémoire.

Annexe A.I : Heat-Inactivation of Fetal and Newborn Sera Did Not Impair the Expansion and Scaffold Engineering Potentials of Fibroblasts

Pellerin, F. A., Caneparo, C., Pellerin, È., Chabaud, S., Pelletier, M., & Bolduc, S. (2021). Heat-Inactivation of Fetal and Newborn Sera Did Not Impair the Expansion and Scaffold Engineering Potentials of Fibroblasts. *Bioengineering (Basel, Switzerland)*, *8*(11), 184. https://doi.org/10.3390/bioengineering8110184

Journal : Bioengineering

Facteur d'impact (2021) : 5,046

Annexe A.II : Bladder cancer cell lines adapt their aggressiveness profile to oxygen tension

Chabaud*, S., Pellerin*, È., Caneparo, C., Ringuette-Goulet, C., Pouliot, F., & Bolduc, S. (2022). Bladder cancer cell lines adapt their aggressiveness profile to oxygen tension. *Oncology letters*, *24*(1), 220. https://doi.org/10.3892/ol.2022.13341

Journal : Oncology Letters

Facteur d'impact (2022) : 3,111

Introduction

La vessie

La vessie est un organe qui fait partie du système urinaire. Ce dernier comprend aussi les reins, les uretères et l'urètre (**Figure 1.1**) (Lanzotti, Tariq, & Bolla, 2022). Les reins et les uretères composent l'appareil urinaire haut, tandis que la vessie et l'urètre composent l'appareil urinaire bas (Libretti & Aeddula, 2022). Ces deux sections se distinguent l'une de l'autre par leur origine embryonnaire. En fait, l'appareil urinaire haut se développe à partir du mésoderme, tandis que l'appareil urinaire bas à partir de l'endoderme (Libretti & Aeddula, 2022). La vessie correspond à un sac musculaire, dont le rôle est d'emmagasiner l'urine jusqu'à plusieurs heures (Lewis, 2000), afin d'avoir des mictions espacées et contrôlées. L'urine est produite par les reins, qui filtrent le sang afin d'assurer l'homéostasie de l'organisme et d'éliminer les déchets présents dans le corps (Lanzotti et al., 2022). L'urine s'écoule ensuite du rein vers la vessie, via les uretères. Lors de la miction, il y a contraction de la vessie et ouverture des sphincters, ce qui permet l'évacuation de l'urine via l'urètre (Lanzotti et al., 2022).

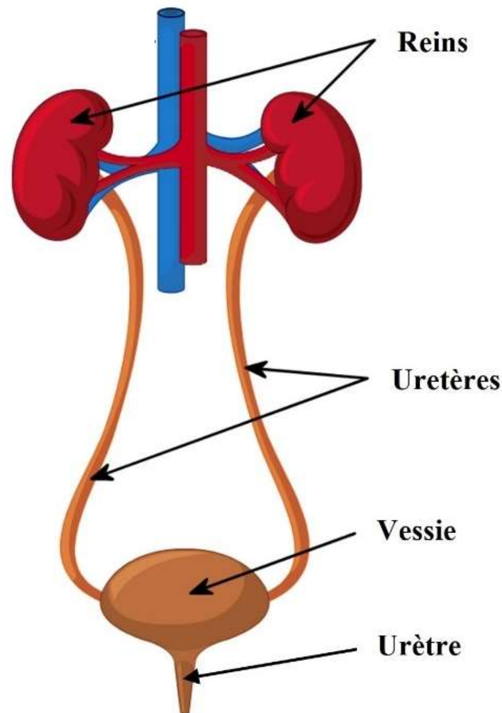

Figure 1.1. Le système urinaire. Un schéma du système urinaire est représenté ici avec les différentes composantes de celui-ci, soit les reins, les uretères, la vessie et l'urètre. Adaptée de (Nagwa, 2022).

La paroi interne de la vessie est composée d'un épithélium pseudostratifié, appelé urothélium, composé de trois couches différenciées, soit la couche basale, la couche intermédiaire et la couche superficielle (Bolla, Odeluga, & Jetti, 2022). La vidange et le remplissage de la vessie provoquent des modifications au niveau de l'urothélium, afin de permettre un ajustement en fonction du volume d'urine contenu (Bolla et al., 2022). L'urothélium de la vessie est entre autres caractérisé par la présence de cellules parapluies au niveau de la couche superficielle, qui correspondent à des cellules binucléées qui protègent les cellules urothéliales sous-jacentes des substances toxiques contenues dans l'urine (Bolla et al., 2022). Les cellules parapluies sont recouvertes d'une couche de glycoprotéines appelées uroplakines, qui forment une plaque qui recouvre et protège chacune des cellules parapluies (Bolla et al., 2022; Matuszewski et al., 2016). En effet, l'urine contient diverses substances délétères pour les cellules, dont l'urée et certains carcinogènes tels que des nitroamines et des thioéthers (Hecht, 2002). En cas de lésion ou de blessure au niveau de la

couche superficielle de la paroi vésicale, les cellules profondes des couches inférieures se différencient afin de remplacer les cellules endommagées à la surface (C. Wang, Ross, & Mysorekar, 2017). Ceci permet de maintenir l'imperméabilité de la vessie afin d'éviter toute fuite interne d'urine dans l'organisme. La vessie peut être affectée par plusieurs pathologies, congénitales et acquises. Parmi les pathologies acquises, il est possible de développer un cancer de la vessie.

Le cancer de la vessie

Le cancer de la vessie (CaV) est le 5e cancer le plus diagnostiqué au Canada. Il s'agit du 4e cancer le plus commun chez l'homme, et le 8e chez la femme. En 2021, au Canada, le CaV représenta 12 500 nouveaux cas estimés, et 2 600 décès associés. Dans la population générale, les probabilités de développer un CaV sont de 1 sur 34, soit 1 sur 22 chez les hommes et 1 sur 73 chez les femmes (Canadian Cancer Society, 2021). Le taux de survie sur 5 ans des patients atteints d'un CaV est évalué à 77% chez les hommes et 75% chez les femmes (Canadian Cancer Society, 2022). Aux États-Unis, cette pathologie représente 81 180 nouveaux cas estimés en 2022, dont 17 100 décès associés, et il s'agit du 4e cancer le plus diagnostiqué chez l'homme, et le 11e chez la femme (Siegel, Miller, Fuchs, & Jemal, 2022).

Le CaV est donc plus fréquent chez l'homme, mais est souvent plus agressif chez la femme (Shariat et al., 2010). Des études ont démontré que les femmes présentent souvent des formes plus avancées de la maladie, ainsi associées à un moins bon pronostic. En effet, une étude a démontré que les femmes étaient plus susceptibles de développer un CaV multifocal et de plus grosses tumeurs (Shariat et al., 2010). Les différences observées entre les deux sexes pourraient être causées par la présence de différents facteurs de risque et par la régulation des hormones sexuelles (Dobruch et al., 2016). Toutefois, malgré le fait de standardiser en fonction de l'exposition à des facteurs carcinogènes, comme le tabagisme et les composés chimiques dans le cadre du travail, ce cancer reste prédominant chez les hommes, ce qui suggère l'implication de facteurs intrinsèques qui pourraient jouer un rôle important dans la pathologie du CaV (Mizushima, Tirador, & Miyamoto, 2017). Ce sont majoritairement les

personnes plus âgées qui sont affectées par ce type de cancer. En fait, 9 patients sur 10 sont âgés de plus de 55 ans, et la moyenne d'âge au moment du diagnostic de CaV est de 73 ans (Siegel, Miller, & Jemal, 2019).

Le facteur de risque principal du CaV est le tabagisme. Des études ont démontré qu'un fumeur était jusqu'à sept fois plus à risque de développer un CaV comparativement à un non-fumeur (American Society of Clinical Oncology, 2021). Les produits issus de la combustion du tabac jouent donc un rôle majeur dans cette pathologie. Ces risques augmentent de manière plus importante avec le nombre d'années de tabagisme. Il est estimé que près de 50% des cas de CaV sont associés au tabagisme (Janković & Radosavljević, 2007). Bien que la vessie soit adaptée aux produits toxiques et aux déchets produits de manière physiologique par l'organisme, la consommation du tabac libère des produits toxiques non physiologiques, comme l'arsenic, le formaldéhyde, le benzo[a]pyrène et l'ammoniaque (Morgan, Byron, Baig, Stepanov, & Brewer, 2017). Ces substances délétères vont être excrétées via l'urine et puisque la vessie emmagasine habituellement l'urine pendant plusieurs heures, l'urothélium vésical est donc exposé de manière chronique à ces produits toxiques (Lewis, 2000). Les autres facteurs de risque principaux associés à ce cancer sont l'âge, le sexe, l'exposition à certains composés chimiques et les infections urinaires chroniques (DeGeorge, Holt, & Hodges, 2017; Metts, Metts, Milito, & Thomas, 2000). Comme mentionné précédemment, ce cancer affecte principalement des patients plus âgés, et majoritairement des hommes. Des études ont démontré que l'exposition à certains composés pouvait augmenter le risque de développer un CaV. Parmi ces substances, il est possible de mentionner l'usage d'amines aromatiques et les hydrocarbures aromatiques polycycliques, tels le naphtylamine, le naphtalène et le benzo[a]pyrène (Janković & Radosavljević, 2007). Certaines professions sont associées à des risques plus importants de CaV à cause de l'exposition à ces composés toxiques, comme les peintres, l'industrie du métal, l'industrie du cuir, les coiffeuses et barbiers, le personnel de l'industrie du textile, et les activités en lien avec la production de l'aluminium (Janković & Radosavljević, 2007). Finalement, des études ont démontré que les infections urinaires chroniques constituaient un facteur de risque pour le CaV. En fait, la présence de cystites récidivantes provoquerait une irritation chronique de l'urothélium, menant à un métabolisme cellulaire augmenté et résultant en la libération accrue de

métabolites carcinogènes (Janković & Radosavljević, 2007). Le symptôme principal associé au CaV est l'hématurie, soit la présence de sang dans les urines. Ce symptôme serait présent chez 90% des patients (Metts et al., 2000). Parmi les autres symptômes fréquemment retrouvés, il est possible de mentionner la pollakiurie, soit une fréquence de miction élevée, la dysurie, soit la difficulté à uriner, et l'obstruction urétrale (Metts et al., 2000).

Le CaV peut se présenter sous deux formes principales, soit la forme non-invasive et la forme invasive (**Figure 1.2**) (Metts et al., 2000). La forme non-invasive est caractérisée par la présence de cellules cancéreuses confinées dans la muqueuse vésicale, et affecte donc les cellules urothéliales. Cette forme touche 90% des patients, et est caractérisée par un taux de récidive élevé. En fait, le taux de récidive sur 5 ans est de 50 à 70%, et 10% des cancers non-invasifs progressent vers un cancer invasif sur 5 ans (Metts et al., 2000). Le traitement principal pour un CaV non-invasif est la résection transurétrale, qui correspond à une intervention chirurgicale où la tumeur vésicale est retirée par cystoscopie via l'urètre (DeGeorge et al., 2017). Cette intervention est habituellement accompagnée d'un traitement intravésical d'immunothérapie ou de chimiothérapie (DeGeorge et al., 2017). La forme invasive est caractérisée par la présence de cellules cancéreuses qui envahissent le muscle de la vessie, appelé détrusor. En présence d'un CaV invasif, la cystectomie radicale, soit le retrait complet de la vessie, est l'intervention de choix. Cette intervention est habituellement couplée à un traitement de chimiothérapie afin d'améliorer le taux de survie (DeGeorge et al., 2017). En effet, quand la prise en charge d'un CaV invasif ne comprend qu'un traitement local, la majorité des patients développent des métastases en deux ou trois ans. En effet, la forme invasive avec métastases est une forme de CaV qui évolue rapidement, ce qui résulte en un taux de survie de moins de 5% sur 5 ans (Metts et al., 2000).

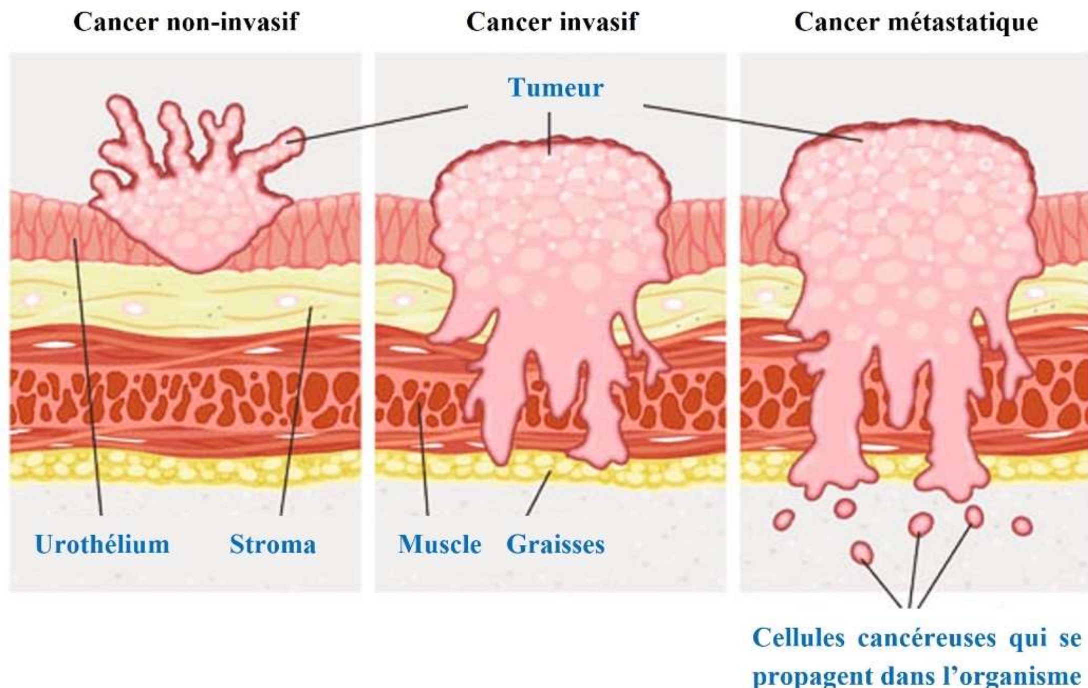

Figure 1.2. Le cancer de la vessie. Un schéma du cancer de la vessie est représenté ici avec les principales formes, soit la forme non-invasive, la forme invasive et le cancer métastatique. Adaptée de (CxBladder, 2022).

Comme mentionné précédemment, le CaV est prédominant chez l'homme, mais est généralement présent sous forme plus agressive chez la femme. Bien que la vessie ne soit pas reconnue comme étant un tissu hormono-sensible, des études ont démontré le rôle des récepteurs hormonaux, tels que les récepteurs des estrogènes (ERα et ERβ) et les récepteurs des androgènes (AR), dans l'initiation et la progression du CaV (Godoy, Gakis, Smith, & Fahmy, 2016; P. Li, Chen, & Miyamoto, 2017). Des métabolites estrogéno-mimétiques ont été identifiés dans l'urine des patients infectés par le parasite *Schistosoma haematobium*, pour lesquels l'activation résultante des ERα favorisait la prolifération des cellules cancéreuses de vessie (Bernardo et al., 2020). D'un côté, des études semblent montrer que le récepteur ERα inhiberait l'invasion du CaV en diminuant les niveaux d'ARN circulaires, tel que circ_0023,642, et en augmentant les niveaux du micro-ARN miR-490-5p, ce qui provoquerait une diminution de l'expression du récepteur du facteur de croissance épidermique (EGFR) (Wu et al., 2019). D'un autre côté, l'expression du récepteur ERβ

semble être augmenté dans les cancers de stade avancé, et est exprimé plus fortement dans le CaV invasif (Miyamoto et al., 2012; Moorthy, Prabhu, & Venugopal, 2020). Le récepteur ERβ favorise la croissance tumorale et l'invasion du CaV via une augmentation de la synthèse de miR-92a et une diminution de l'expression du suppresseur de tumeur DAB2IP (Ou et al., 2018). L'activation des récepteurs ERβ et AR corrèle avec l'inactivation du suppresseur de tumeur FOXO1 qui est inhibé dans le CaV, ce qui induirait une carcinogenèse au niveau des cellules urothéliales et une croissance tumorale (Ide et al., 2020). Le récepteur ERβ jouerait aussi un rôle protecteur envers les cellules cancéreuses de vessie en modulant l'apoptose (Goto et al., 2020). Les récepteurs hormonaux joueraient aussi un rôle dans les effets du traitement (Creta et al., 2021) et le pronostic (Moorthy et al., 2020) chez les patients atteints d'un CaV. En fait, des études ont démontré que l'activation du récepteur ERβ pourrait induire une résistance au cisplatine, un médicament chimiothérapeutique utilisé dans le traitement du CaV, via l'activation des β-caténines (Goto et al., 2020). Des études ont de plus démontré que d'autres cellules du microenvironnement tumoral, soit les fibroblastes associés au cancer (CAF), pouvaient induire une résistance au cisplatine chez les cellules cancéreuses de vessie, via la voie de signalisation du récepteur ERβ (Long et al., 2019). Une masse cancéreuse est donc un système complexe constitué de différents types cellulaires pouvant interagir entre eux et moduler la croissance et la progression tumorale. La compréhension de ce microenvironnement tumoral est essentielle afin d'identifier les éléments pouvant influencer le développement du cancer de la vessie.

Le microenvironnement tumoral

Le microenvironnement tumoral est constitué par un ensemble de cellules, incluant les cellules épithéliales, les fibroblastes, les cellules immunitaires et les cellules endothéliales des vaisseaux sanguins, ainsi que la matrice extracellulaire (Anderson & Simon, 2020).

Les cellules épithéliales qui acquièrent des mutations peuvent mener au développement de cellules cancéreuses (American Society of Clinical Oncology, 2019). D'un côté, les cellules cancéreuses non-invasives issues de l'épithélium ne peuvent pas passer la membrane basale afin d'atteindre le stroma. De l'autre côté, les cellules cancéreuses invasives peuvent pénétrer

dans la matrice extracellulaire, afin de rejoindre la circulation sanguine pour former des métastases dans l'organisme (Kalluri & Weinberg, 2009). La transition d'un phénotype non-invasif vers un phénotype invasif nécessite plusieurs étapes, dont une étape cruciale qui correspond à la transition épithélio-mésenchymateuse (EMT). L'EMT est un processus complexe, impliquant plusieurs changements biochimiques, au cours duquel une cellule épithéliale perd sa polarité (Kalluri & Weinberg, 2009). Habituellement, la polarité d'une cellule épithéliale lui permet d'interagir avec la membrane basale via son extrémité basale. Ainsi, la perte de la polarité cellulaire permet à la cellule épithéliale d'acquérir un phénotype mésenchymateux (Kalluri & Weinberg, 2009). À la suite de l'EMT, la cellule cancéreuse possède des capacités migratoires et invasives augmentées, est plus résistante à l'apoptose et produit de manière accentuée des composantes de la matrice extracellulaire (Kalluri & Weinberg, 2009). L'EMT est considérée complète lorsque la membrane basale sous-jacente à la cellule épithéliale a été désintégrée, et que cette dernière peut migrer à distance de la barrière épithéliale dans le stroma (Kalluri & Weinberg, 2009).

Les fibroblastes présents dans le stroma sont responsables de la production de la matrice extracellulaire. La matrice extracellulaire contient plusieurs composantes, dont le collagène, la fibronectine, l'élastine, la laminine et des protéoglycanes (Yue, 2014). La production de matrice par les fibroblastes est essentielle au renouvellement et à la réparation tissulaire (Bainbridge, 2013). En fait, les cellules de l'épithélium et celles du stroma communiquent et interagissent entre elles (Whiteside, 2008). Une matrice extracellulaire appropriée permet la différenciation adéquate de l'urothélium vésical, ce qui assure l'étanchéité et la fonction barrière de la vessie (Bouhout, Chabaud, & Bolduc, 2016; Hudson, Carmean, & Bassuk, 2007). Les fibroblastes peuvent, entre autres, interagir avec les cellules cancéreuses, ce qui peut mener à l'induction des fibroblastes en fibroblastes associés au cancer (CAF). Les CAF jouent un rôle critique dans la progression tumorale. Des études ont démontré que les cellules cancéreuses invasives de vessie libèrent du TGF-β, ce qui provoque l'induction des fibroblastes vésicaux sains en CAF (Ringuette Goulet et al., 2018). Une fois induits, les CAF libèrent à leur tour de l'interleukine-6 (IL-6) dans l'environnement extracellulaire, ce qui peut augmenter l'EMT des cellules cancéreuses non-invasives de vessie (Goulet et al., 2019). Ainsi, l'interaction entre les CAF et les cellules cancéreuses pourrait permettre à ces

dernières d'envahir le stroma. Toutefois, les CAF peuvent à la fois promouvoir ou inhiber la croissance tumorale (Anderson & Simon, 2020). Tout comme les fibroblastes, les CAF produisent de la matrice extracellulaire, des facteurs de croissance et des cytokines. Ceci permet un remodelage de la matrice afin de supporter la croissance tumorale. Les CAF induisent une rigidification de la matrice extracellulaire, ce qui active des voies de signalisation pro-tumorales, telles que les voies impliquant la phosphoinositide 3-kinase (PI3K) et la kinase régulée par signaux extracellulaires (ERK) associées à la survie et la prolifération cellulaire (Najafi, Farhood, & Mortezaee, 2019). La rigidification de la matrice extracellulaire résulte, entre autres, de l'accumulation d'acide hyaluronique, de collagène et de fibronectine (Najafi et al., 2019). Ce phénomène favorise ainsi la prolifération, la migration et l'invasion des cellules cancéreuses. Au niveau clinique, la rigidification de matrice tumorale par les CAF est associée à un taux élevé de métastases et un mauvais pronostic (Najafi et al., 2019). De plus, les CAF peuvent libérer des cytokines anti-inflammatoires afin d'inhiber les cellules immunitaires (Anderson & Simon, 2020).

Les cellules immunitaires sont une composante importante du microenvironnement tumoral. Selon le contexte, les cellules immunitaires peuvent inhiber la croissance tumorale ou encore la stimuler (Anderson & Simon, 2020). En fait, les cellules immunitaires, comme les lymphocytes T cytotoxiques, peuvent reconnaître les antigènes tumoraux présents à la surface des cellules cancéreuses afin de les éliminer. Toutefois, dans le cas d'une infection chronique, des études ont démontré que l'inflammation persistante était un mécanisme sous-jacent du développement tumoral (Anderson & Simon, 2020).

Pour leur part, les cellules endothéliales forment les vaisseaux sanguins. Ces derniers permettent, entre autres, l'apport en eau et en nutriments, le maintien de l'homéostasie métabolique et le transport des cellules immunitaires. Afin de surmonter l'hypoxie et l'acidification du milieu extracellulaire dues à la croissance tumorale, la tumeur induit la formation d'une nouvelle branche du réseau sanguin en stimulant la migration de cellules endothéliales (Anderson & Simon, 2020). Ces nouveaux vaisseaux sanguins assurent un apport en oxygène et en nutriments à la tumeur. Ces vaisseaux jouent aussi un rôle important dans la formation de métastases. En effet, les cellules cancéreuses qui envahissent le stroma

peuvent utiliser le processus d'intravasation. Durant ce processus, la cellule cancéreuse adhère aux cellules endothéliales, puis migre entre deux cellules afin d'atteindre la circulation sanguine pour potentiellement former des tumeurs à distance (Anderson & Simon, 2020).

Les différents types cellulaires présents dans le microenvironnement tumoral communiquent entre eux et sont ainsi essentiels à la croissance et à la progression tumorale. Afin d'assurer l'EMT, la prolifération et la migration des cellules cancéreuses, ainsi que le remodelage matriciel par les fibroblastes, les cellules du microenvironnement tumoral nécessitent un métabolisme énergétique adapté.

Le métabolisme énergétique

Le métabolisme énergétique est un processus biologique qui permet la synthèse d'énergie, sous forme d'adénosine triphosphate (ATP), à partir de nutriments afin de permettre la croissance cellulaire (Rigoulet et al., 2020). Le métabolisme énergétique cellulaire comprend deux voies principales, soit la glycolyse et la respiration mitochondriale.

La glycolyse est une voie métabolique qui convertit une molécule de glucose en deux molécules de pyruvate dans le cytoplasme pour produire de l'ATP (X. B. Li, Gu, & Zhou, 2015). La glycolyse est un processus qui comprend un total de dix étapes, dont chacune des réactions chimiques est catalysée par une enzyme spécifique (**Figure 1.3**). Au terme de ce processus, la glycolyse consomme deux molécules d'ATP pour former deux molécules de NADH et quatre molécules d'ATP (X. B. Li et al., 2015).

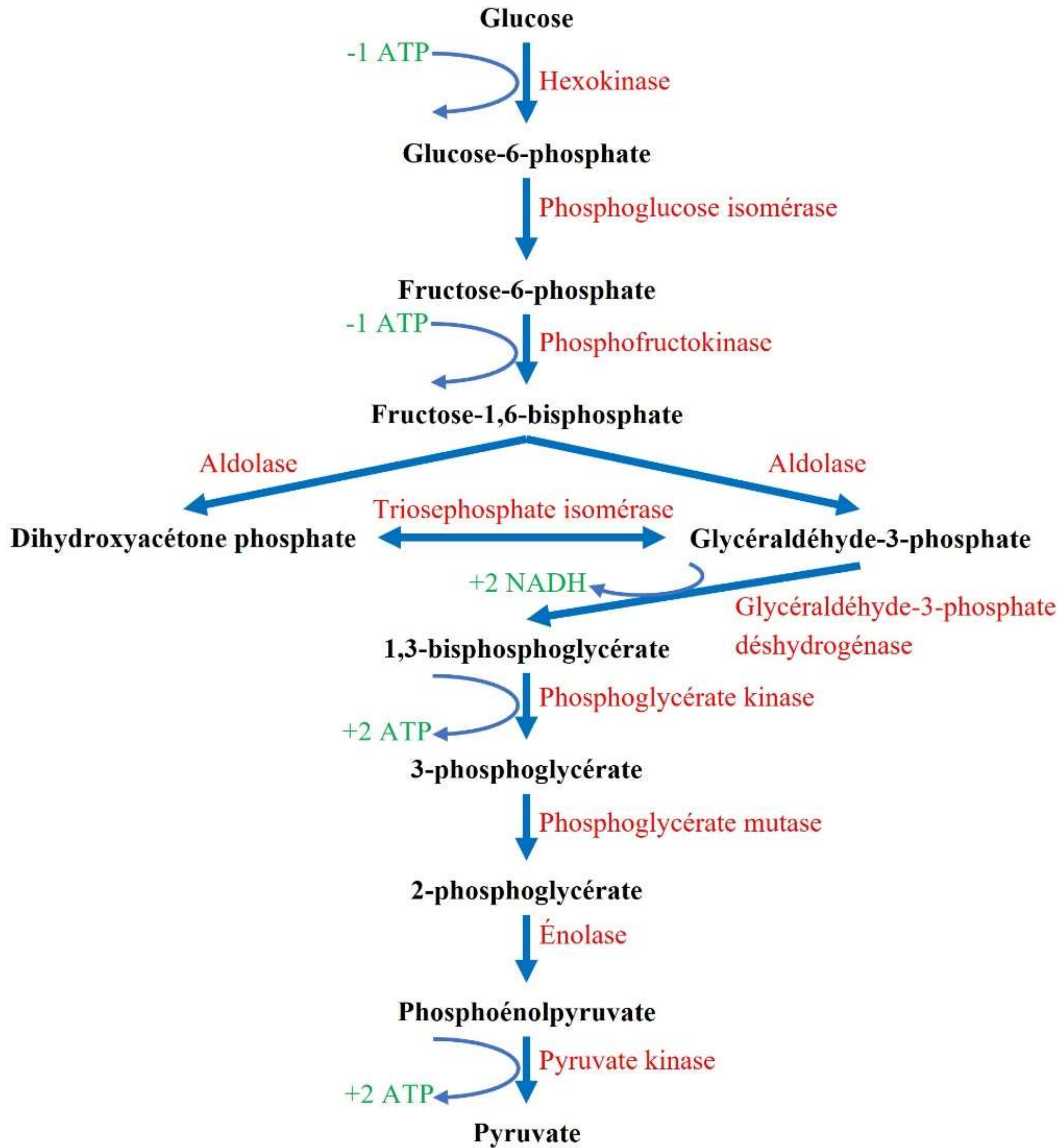

Figure 1.3. La glycolyse. Un schéma de la glycolyse représentant les dix étapes et les enzymes spécifiques associées à chacune des étapes, ainsi que la production de molécules énergétiques.

Les intermédiaires produits au cours de la glycolyse peuvent servir à d'autres voies métaboliques (X. B. Li et al., 2015), par exemple la voie des pentoses phosphate. En condition anaérobie, soit en absence d'oxygène, le pyruvate est converti en lactate par les enzymes lactates déshydrogénases. Les cellules cancéreuses privilégient habituellement la voie des lactates déshydrogénases, malgré la présence d'oxygène (X. B. Li et al., 2015). En

condition aérobie, soit en présence d’oxygène, le pyruvate est utilisé lors de la respiration mitochondriale où il est dégradé en dioxyde de carbone (CO_2) pour produire de l’énergie (X. B. Li et al., 2015). Le pyruvate peut aussi être utilisé par plusieurs autres voies métaboliques. En fait, le pyruvate peut être redirigé vers la voie de la gluconéogenèse afin de régénérer du glucose. Le pyruvate peut aussi être utilisé pour produire de l’acétyl-CoA afin de synthétiser des acides gras. Finalement, le pyruvate peut être utilisé pour synthétiser certains acides aminés, comme l’alanine (X. B. Li et al., 2015).

La respiration mitochondriale comprend deux phases principales, soit le cycle de Krebs (aussi appelé cycle de l’acide citrique) et la chaine de transport d’électrons (aussi appelée phosphorylation oxydative). Avant d’entrer dans le cycle de Krebs, le pyruvate issu de la glycolyse est transporté dans la mitochondrie puis y est converti en acétyl-CoA (Alabduladhem & Bordoni, 2022). L’acétyl-CoA peut ensuite être catabolisé en trois molécules de CO_2 à travers les huit étapes du cycle de Krebs, afin de fournir des molécules à haute teneur en énergie (**Figure 1.4**) (Alabduladhem & Bordoni, 2022). À partir d’une molécule d’acétyl-CoA, le cycle de Krebs permet de produire trois molécules de NADH, une molécule de $FADH_2$ et une molécule de GTP. Les molécules de NADH et de $FADH_2$ produites lors de la glycolyse et du cycle de Krebs sont oxydées par la chaîne de transport d’électrons pour produire de l’ATP (Alabduladhem & Bordoni, 2022). De plus, le cycle de Krebs produit plusieurs intermédiaires qui servent de précurseurs pour différents processus cataboliques et anaboliques, par exemple la synthèse d’acides aminés et de stéroïdes (Alabduladhem & Bordoni, 2022).

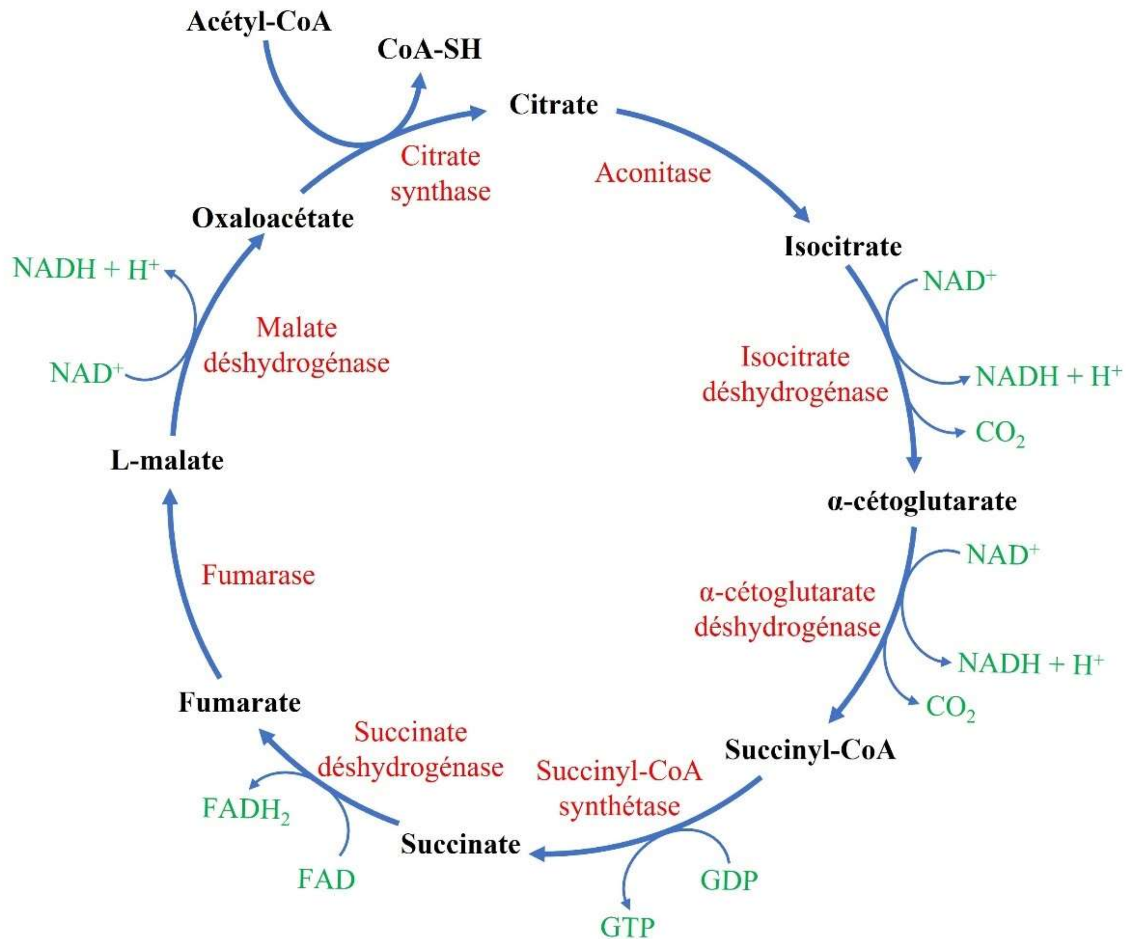

Figure 1.4. Le cycle de Krebs. Un schéma du cycle de Krebs, correspondant à la première phase de la respiration mitochondriale, représentant les huit étapes et les enzymes spécifiques associées à chacune des étapes, ainsi que la production de molécules énergétiques.

La chaine de transport d'électrons est pour sa part composée de cinq complexes protéiques (**Figure 1.5**). D'un côté, la molécule de NADH transfère ses deux électrons au complexe I, aussi appelé ubiquinone oxydoréductase (Ahmad, Wolberg, & Kahwaji, 2022). Les électrons sont ensuite transférés à la coenzyme Q (ubiquinone). Durant cette étape, quatre protons (ions hydrogène) sont pompés dans l'espace intermembranaire de la mitochondrie (Ahmad et al., 2022). D'un autre côté, le complexe II, aussi appelé succinate déshydrogénase, accepte les électrons provenant du $FADH_2$ et agit comme deuxième point d'entrée pour la chaine de transport d'électrons (Ahmad et al., 2022). Ensuite, les électrons de l'ubiquinone sont

transférés au complexe III, aussi appelé cytochrome c réductase, puis transférés au cytochrome c. Durant cette étape, le complexe III pompe également quatre protons dans l'espace intermembranaire de la mitochondrie, ce qui contribue au gradient électrochimique (Ahmad et al., 2022). Par la suite, le cytochrome c transfère ses électrons au complexe IV, aussi appelé cytochrome c oxydase. Le complexe IV transfère à son tour les électrons à l'oxygène, qui agit comme dernier transporteur d'électrons. L'oxygène est ensuite couplé avec deux protons afin de former une molécule d'eau (H_2O) (Ahmad et al., 2022). Durant cette étape, quatre protons supplémentaires sont pompés dans l'espace intermembranaire. Finalement, le complexe V, appelé ATP synthase, utilise le gradient électrochimique généré par la chaine de transport d'électrons pour produire de l'ATP (**Figure 1.5**) (Ahmad et al., 2022). Chaque molécule de NADH génère trois molécules d'ATP, tandis que chaque molécule de $FADH_2$ génère deux molécules d'ATP (Cooper, 2000). Ainsi, l'oxydation complète d'une molécule de glucose en condition aérobie permet de générer un total de 36 molécules d'ATP (deux molécules d'ATP générées via la glycolyse et 34 molécules d'ATP générées via la respiration mitochondriale) (Cooper, 2000).

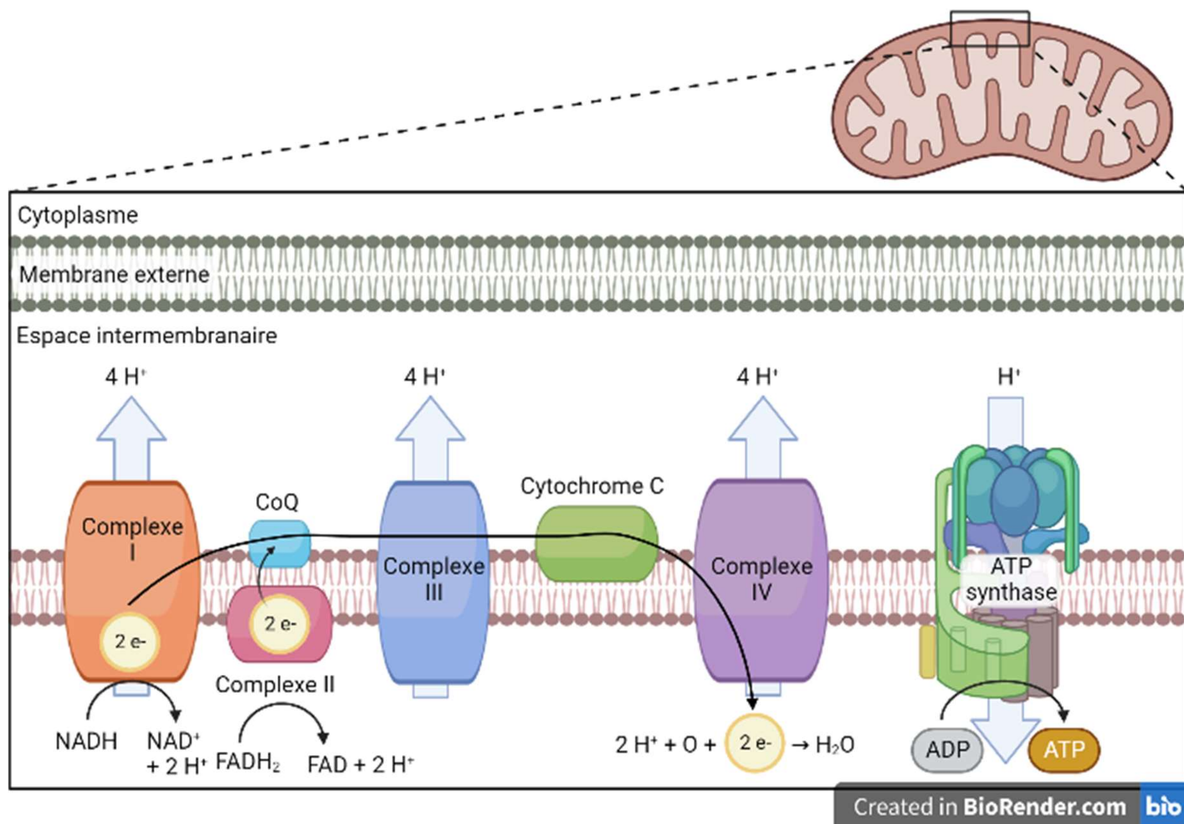

Figure 1.5. La chaîne de transport d'électrons. Un schéma de la chaîne de transport d'électrons, correspondant à la deuxième phase de la respiration mitochondriale, représentant les cinq complexes protéiques, ainsi que les substrats énergétiques utilisés afin d'établir un gradient électrochimique permettant la synthèse d'ATP. Créée avec BioRender.com.

Effet de Warburg

Dans les années 1920, Otto Warburg et ses collaborateurs ont observé que les tumeurs consommaient énormément de glucose comparativement aux tissus environnants (Otto Warburg, 1925). De plus, ils ont remarqué que le glucose était fermenté en lactate, malgré la présence d'oxygène, et que la respiration aérobie seule pouvait soutenir la viabilité tumorale (O. Warburg, Wind, & Negelein, 1927). Le phénomène observé fut nommé effet de Warburg dans les années 1970 par Efraim Racker (Liberti & Locasale, 2016). De nos jours, l'effet de Warburg est connu comme étant une altération du profil métabolique des cellules cancéreuses correspondant à une des caractéristiques principales des tumeurs. L'effet de Warburg correspond à une reprogrammation métabolique à partir d'un métabolisme oxydatif vers un

métabolisme glycolytique (Liberti & Locasale, 2016). La glycolyse est ainsi favorisée, au détriment de la respiration mitochondriale, malgré la présence d'oxygène et de mitochondries fonctionnelles. Cette reprogrammation métabolique permet aux cellules cancéreuses de supporter leur croissance et leur prolifération cellulaire rapide, en plus de favoriser l'invasion du stroma (Liberti & Locasale, 2016).

La glycolyse aérobie permet aux cellules cancéreuses de synthétiser plus rapidement de l'ATP, malgré le fait que la production totale d'ATP soit moindre. En fait, la production de lactate à partir de glucose est beaucoup plus rapide que l'oxydation complète d'une même molécule de glucose via les mitochondries (Shestov et al., 2014). La conversion du pyruvate en lactate permet aussi la régénération de NAD+, qui est un élément essentiel à la glycolyse (Liberti & Locasale, 2016). L'effet de Warburg permet aussi la production excessive d'intermédiaires biosynthétiques dans le cytoplasme de la cellule, ce qui permet la synthèse de protéines, de lipides et de nucléotides. La génération de ces composantes permet ainsi de supporter la prolifération importante et incontrôlée des cellules cancéreuses (Vander Heiden, Cantley, & Thompson, 2009). Dans le microenvironnement tumoral, l'effet de Warburg induit une production importante de pyruvate dans les cellules. L'excès de pyruvate est redirigé vers les enzymes intracellulaires lactates déshydrogénases, ce qui mène à la production accrue de lactate. Le lactate produit est exporté à l'extérieur des cellules par les transporteurs de monocarboxylate MCT (Halestrap & Price, 1999), ce qui induit une diminution du pH local et provoque l'acidification du milieu extracellulaire (Liberti & Locasale, 2016). Cette acidification est généralement bénéfique pour les cellules cancéreuses, puisqu'elle permet un remodelage du stroma, ce qui favorise l'invasion tumorale et ainsi la formation de métastases (Estrella et al., 2013; Niu, Luo, Wang, Xia, & Xie, 2021). L'acidification favorise le remodelage du stroma, entre autres, en stimulant la production de métalloprotéinases matricielles (MMP) qui dégradent les composantes de la matrice extracellulaire (Dratkiewicz et al., 2021). De plus, l'accumulation de lactate inhibe les cellules immunitaires, tels que les monocytes, qui sont responsables de l'élimination des cellules cancéreuses (Goetze, Walenta, Ksiazkiewicz, Kunz-Schughart, & Mueller-Klieser, 2011).

Des études ont démontré que les CAF présentaient aussi une reprogrammation métabolique, similaire à l'effet de Warburg (Pavlides et al., 2009). Il a été suggéré que le TGF-β aurait un rôle dans cette reprogrammation métabolique. Ainsi, le TGF-β augmenterait la glycolyse aérobie, modulerait certaines enzymes et augmenterait le stress oxydatif, ce qui affecterait le fonctionnement des mitochondries (Guido et al., 2012; Pavlides et al., 2009; Young et al., 2014). Une étude a démontré que le TGF-β active la voie de signalisation PI3K/protéine kinase sérine-thréonine α (Akt)/cible mécanistique de la rapamycine (mTOR) et favorise l'activation du facteur de transcription ATF4, ce qui induit une reprogrammation métabolique chez les fibroblastes pulmonaires (O'Leary et al., 2020). Le lactate produit par les CAF agit comme molécule chimioattractante et comme métabolite pour les cellules cancéreuses (Pavlides et al., 2009). Ainsi, la production excessive de lactate résultant de la reprogrammation métabolique des CAF permet aux cellules cancéreuses d'augmenter leur consommation d'énergie grâce aux métabolites fournis par les CAF (Pavlides et al., 2009).

Les cellules cancéreuses et les autres composantes du microenvironnement tumoral peuvent être affectées par divers facteurs. Les cancers peuvent, entre autres, être influencés par des molécules chimiques synthétisées par l'humain. Parmi ces composés, on retrouve les bisphénols présents dans les objets de plastique.

Les bisphénols et leurs métabolites

Le bisphénol A (BPA) fut rapporté pour la première fois en 1891 par un chimiste russe (Dianin, 1891), et fut synthétisé pour la première fois en 1905 à la suite de la condensation de l'acétone avec deux équivalents phénol (Jalal, Surendranath, Pathak, Yu, & Chung, 2018). Les propriétés estrogéniques du BPA furent découvertes pendant les années 1930, ce qui a mené à plusieurs études sur ce composé afin de savoir s'il pouvait être utilisé comme estrogène synthétique (Wolstenholme, Rissman, & Connelly, 2011). Ces recherches ont permis de découvrir l'utilité du BPA dans la synthèse des résines époxy. Ainsi, depuis les années 1950, le BPA est utilisé comme monomère synthétique dans la synthèse de plusieurs types de plastiques, par exemple les polyesters, les résines époxy et le polycarbonate (Murata & Kang, 2018; Wolstenholme et al., 2011).

De nos jours, le BPA fait partie de la grande famille des bisphénols, qui contient une multitude d'analogues synthétiques (Pellerin, Caneparo, Chabaud, Bolduc, & Pelletier, 2021). Sa présence dans les plastiques fait en sorte qu'on retrouve ce composé chimique dans plusieurs objets utilisés quotidiennement, comme les bouteilles d'eau, les contenants alimentaires et les reçus de caisse. La principale source de contamination au BPA est la nourriture. Toutefois, il est possible d'en retrouver aussi dans les sols, l'air et les cours d'eau (Rochefort, 2017; Shafei et al., 2018). Ainsi, l'humain y est exposé de manière chronique et continue. Une fois dans l'organisme, le BPA est rapidement métabolisé et excrété sous forme de métabolites, principalement via l'urine (Genuis, Beesoon, Birkholz, & Lobo, 2012). Toutefois, une certaine portion demeure non-métabolisée, ce qui permet la détection et la quantification de BPA dans l'urine et le sérum des enfants et des adultes (Di Donato et al., 2017; Murata & Kang, 2018). En fait, plus de 90% des échantillons d'urine chez l'humain contiennent des quantités mesurables de BPA (Bushnik et al., 2010).

Le BPA a la capacité de se lier à plusieurs récepteurs cellulaires, comme les récepteurs des estrogènes ERα et ERβ, le récepteur des androgènes (AR), le récepteur γ lié aux estrogènes (ERRγ) et le récepteur des estrogènes couplé aux protéines G (GPER) (Murata & Kang, 2018). Ses multiples capacités de liaison font en sorte que le BPA est considéré comme un perturbateur endocrinien, puisqu'il peut altérer de multiples voies de signalisation, et ainsi favoriser, entre autres, le développement tumoral. L'exposition au BPA est associée au développement tumoral, principalement pour les cancers hormono-sensibles, tels que les cancers du sein (Zhang, Liu, Weng, & Wang, 2016) et de la prostate (Ho, Rao, To, Schoch, & Tarapore, 2017). Le BPA active entre autres la voie de signalisation GPER/EGFR/ERK qui stimule la prolifération, la croissance et la migration des cellules cancéreuses du sein (Z. Wang, Liu, & Liu, 2017). Des études ont démontré que le BPA pouvait se lier au récepteur GPER afin de stimuler la migration et la prolifération des cellules cancéreuses du larynx en augmentant l'expression d'IL-6 (S. Li, Wang, Tang, Liu, & Yang, 2017). La liaison du BPA au GPER peut aussi mener à l'activation de la voie de signalisation PI3K/Akt/mTOR qui joue un rôle dans la croissance et la prolifération cellulaire. Cette voie inhibe aussi l'apoptose, via une augmentation de l'expression des facteurs anti-apoptotiques, tel que le lymphome à cellule B (Bcl-2), et une diminution de l'expression des facteurs pro-apoptotiques, tel que la

protéine tumorale p53 (Murata & Kang, 2018). L'activation de cette voie de signalisation par le BPA a entre autres été démontré dans le cancer de l'ovaire (Shafei et al., 2018) et le cancer du sein (Dairkee, Luciani-Torres, Moore, & Goodson, 2013). De plus, l'activation du GPER mène à la production de MMP, telles que les MMP-2 et MMP-9, qui dégradent le collagène de la membrane basale, favorisant ainsi la migration des cellules vers la matrice extracellulaire et la formation de métastases (Jiang, Wilson, Smith, Pierce, & Wiebkin, 1994; Murata & Kang, 2018). Des études ont démontré l'augmentation de la production de MMP par le BPA dans le cancer du larynx (S. Li et al., 2017), du poumon (Shafei et al., 2018) et du sein (Zhang et al., 2016). La voie de signalisation EGFR/ERK peut aussi être activée par le BPA via le récepteur ERRγ, et ainsi favoriser la prolifération des cellules cancéreuses de l'endomètre (Yaguchi, 2019). En plus des voies de signalisation cellulaire, il a été démontré que le BPA pouvait inhiber certaines enzymes impliquées dans le métabolisme (Quesnot, Bucher, Fromenty, & Robin, 2014), affectant ainsi des voies critiques, telle que la respiration mitochondriale (Khan et al., 2016).

Puisque de plus en plus de littérature démontre les caractéristiques pro-tumorigéniques du BPA, plusieurs analogues ont été synthétisés afin de le remplacer. Parmi les alternatives développées, il est possible de mentionner le bisphénol S (BPS), qui possède une structure similaire au BPA. De nos jours, le BPS remplace progressivement le BPA, et est maintenant utilisé dans la synthèse des plastiques à cause de son excellente stabilité (Jia, Sun, Ding, Cao, & Yang, 2018). Initialement, le BPS était considéré plus sécuritaire que le BPA. Toutefois, de plus en plus d'études démontrent les capacités estrogéno-mimétiques de ce composé, ainsi que les conséquences physiologiques associées à son exposition. En fait, il a été démontré que le BPS avait des effets anti-androgéniques et réduisait la sécrétion de testostérone des cellules testiculaires humaines (Eladak et al., 2015). Tout comme le BPA, le BPS peut s'accumuler dans l'organisme par ingestion et par exposition continue. Aux États-Unis, une étude a démontré que 78% des échantillons d'urine contenait du BPS à des concentrations similaires à celles du BPA, soit entre 1 et 100 nM (Song, Fan, Tian, & Wen, 2019). Le BPS peut, entre autres, se lier aux récepteurs ERα/β et GPER, ce qui favorise la croissance et la migration des cellules cancéreuses de poumon, via une augmentation de l'expression de TGF-β (Song et al., 2019). Le BPS peut se lier au récepteur ERRα des cellules cancéreuses

neuroendocrines, stimulant ainsi leur migration et invasion (Jia et al., 2018). Plusieurs études suggèrent donc que le BPS semble être un perturbateur endocrinien, dont l'exposition mène à des conséquences similaires à celles associées au BPA, dont le développement tumoral (Song et al., 2019).

À la suite de leur ingestion et absorption par l'organisme, le BPA et le BPS sont habituellement rapidement métabolisés par le foie. La demi-vie du BPA est d'environ quatre à cinq heures chez l'adulte. Toutefois, son métabolisme est plus faible chez les fœtus et les enfants (Gore et al., 2015), ce qui peut résulter en une exposition plus importante. Le BPA et le BPS sont convertis en différents métabolites, dont le principal est sous forme conjuguée avec un acide glucuronique, ce qui forme un bisphénol glucuronidé (Gramec Skledar & Peterlin Mašič, 2016). Le deuxième métabolite le plus fréquent est sous forme conjuguée avec un sulfate (Gramec Skledar & Peterlin Mašič, 2016; K. L. Ho et al., 2017). Près de 99% du BPA absorbé est métabolisé par le foie, tandis que seulement 41% du BPS absorbé est converti en métabolites (Gayrard et al., 2019). Ces composés sont principalement excrétés par l'urine, et parfois via la sueur (Genuis et al., 2012). Cependant, des études ont démontré que certains organes, tels que les poumons, le foie et les reins, possèdent une enzyme appelée β-glucuronidase qui a la capacité de déconjuguer le BPA afin de libérer la molécule originale dans l'organisme (Genuis et al., 2012). De plus, malgré le fait d'avoir été métabolisés, les métabolites des bisphénols peuvent demeurer actifs dans plusieurs organes (Thoene, Dzika, Gonkowski, & Wojtkiewicz, 2020).

Problématique, hypothèse et objectifs du projet de recherche

La vessie n'est pas reconnue comme étant un tissu hormono-sensible. Toutefois, le rôle de cet organe est de stocker l'urine, qui contient du BPA et du BPS (Bushnik et al., 2010; Song et al., 2019). Il a été démontré que les récepteurs hormonaux, tels que les ER et le AR, jouaient un rôle dans l'initiation et la progression du cancer de la vessie (Godoy et al., 2016; P. Li et al., 2017). Ainsi, les bisphénols présents dans l'urine pourraient se lier aux récepteurs hormonaux de l'urothélium, et ainsi jouer un rôle dans le développement du cancer de la vessie. Nous avons émis l'hypothèse que l'exposition chronique à des concentrations physiologiques de BPA ou de BPS allait altérer le métabolisme énergétique et l'activité physiologique des cellules urothéliales saines, des cellules cancéreuses de vessie, des fibroblastes vésicaux et des fibroblastes associés au cancer, favorisant ainsi le développement et la progression du cancer de la vessie.

Afin de vérifier ou d'infirmer cette hypothèse, nous avons établi deux objectifs. Dans un premier temps, nous avons voulu déterminer l'impact d'une exposition chronique à des concentrations physiologiques de BPA ou de BPS sur le métabolisme énergétique et l'activité physiologique des cellules urothéliales saines et des cellules cancéreuses de vessie. Dans un deuxième temps, nous avons voulu déterminer l'impact d'une exposition chronique à des concentrations physiologiques de BPA sur le métabolisme énergétique des fibroblastes vésicaux et des fibroblastes associés au cancer. Ses travaux devraient apporter des informations novatrices, puisqu'aucune étude n'a précédemment étudié les liens entre l'exposition aux bisphénols et le cancer de la vessie. Nos recherches permettront ainsi une meilleure compréhension des conséquences de l'exposition chronique aux bisphénols sur le développement et la progression du cancer de la vessie, ainsi que les effets cliniques associés.

Chapitre 1 : Les effets de perturbateurs endocriniens des bisphénols sur les cancers urologiques

1.1 Résumé

Les bisphénols sont des perturbateurs endocriniens qui peuvent moduler les voies de signalisation hormonale. Ces composés peuvent se lier aux récepteurs stéroïdiens exprimés par la prostate et la vessie. Leur liaison aux récepteurs hormonaux peut altérer les fonctions physiologiques normales des cellules. Récemment, l'exposition aux bisphénols a été reliée à la progression de certains cancers. Des études suggèrent que les perturbateurs endocriniens pourraient, entre autres, mener au développement du cancer de la prostate. De plus, les bisphénols sont retrouvés dans l'urine de la majorité des humains. Leur présence dans l'urine suggère qu'ils pourraient affecter la physiologie normale de la vessie et ainsi mener au développement du cancer de la vessie. Cette revue de la littérature se concentrera sur les cancers de prostate et de vessie, deux cancers urologiques qui partagent des processus carcinogènes similaires. Cette revue littéraire pourrait aider à établir les rôles putatifs des bisphénols sur la santé publique.

1.2 Abstract

Bisphenols are endocrine-disrupting chemicals found in a broad range of products that can modulate hormonal signalling pathways and various other biological functions. These compounds can bind steroid receptors, e.g. estrogen and androgen receptors, expressed by numerous cells and tissues, including the prostate and the bladder, with the potential to alter their homeostasis and normal physiological functions. In the past years, exposure to bisphenols was linked to cancer progression and metastasis. As such, recent pieces of evidence suggest that endocrine-disrupting chemicals can lead to the development of prostate cancer. Moreover, bisphenols are found in the urine of the wide majority of the population. They could potentially affect the bladder's normal physiology and promote cancer development, even if the bladder is not recognized as a hormone-sensitive tissue. This review will focus on prostate and bladder malignancies, two urological cancers that share standard carcinogenic processes. The description of the underlying mechanisms involved in cell toxicity, and the possible roles of bisphenols in the development of prostate and bladder cancer, could help establish the putative roles of bisphenols on public health.

1.3 Article

Endocrine-disrupting effects of bisphenols on urological cancers

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Running head: Bisphenols and urological cancers

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1.3.1 Introduction

Since the 1980s, industrialized countries have witnessed an increased incidence of many different types of cancers, including hormone-dependent cancers such as breast [1], testicular [2, 3] and prostate cancer (PCa) [4]. The American Cancer Society estimated that more than 1.7 million new cancers would be diagnosed in 2019, with approximately 607,000 cancer-associated deaths [5], compared to 1.1 million estimated new cases in 1979, with about 395,000 cancer-associated deaths [6]. This increase can be associated with better detection techniques and the ageing of the population. Nevertheless, other factors are often underestimated, such as the increasing number of endocrine disruptors (EDs) found in our environment [7]. It is especially true for bisphenols (BPs), which are found in a broad range of plastic products. Exposure to BPs is linked to cancer progression and metastasis. This review focused on urological cancers, PCa and bladder cancer (BCa), which share standard carcinogenic processes. Studies have shown the potential role of BPs in PCa development through the activation of estrogen (ER) and androgen (AR) receptors and downstream pathways. The bladder is not recognized as hormone-sensitive tissue, but its role is to store urine, in which BPs are found for >90% of the population [8]. With the presence of hormone receptors on urothelial cells, the epithelial cells of the bladder, BPs could also play a role in BCa development.

1.3.1.1 Endocrine disruptors

EDs are molecules that can modulate the endocrine system, which controls and regulates hormones' production and activity. EDs can, therefore, alter the expression of intra- and extracellular receptors, as well as affect their activity and their associated pathways. This disruption can have substantial consequences on other systems and biological functions, such as reproduction, fetal development and the immune response [9]. Exposure to EDs can also lead to the development of hormone-dependent cancers. EDs can interact with components of endocrine signalling pathways such as ERs. These receptors are responsible for modulating estrogen synthesis and associated enzyme activity or indirect regulation of transcription factors [10]. Studies have identified the presence of ERα mainly in estrogen-targeted tissues such as breast, uterus, testis and ovaries. In contrast, ERβ is primarily

expressed in the prostate, bladder, lung and intestines [11, 12]. This global ER expression suggests that EDs could cause substantial consequences on target organs.

It is possible to distinguish three categories of EDs: physical, biological and chemical (**Figure 2.1**). These different types of EDs can impact signalling pathways in different ways, although they share similar action mechanisms (**Figure 2.2**). Whether EDs are natural or synthetic, they are ubiquitous in our environment. They are found in a broad range of industrial, consumer and medical products such as plastic bottles, food containers, pharmaceutical drugs and pesticides [13]. The constant presence of EDs causes continuous exposure to humans and wildlife since these disrupting molecules are found in soil, dust and water. They are, therefore, inhaled or ingested and can affect the endocrine system [10]. Since EDs are omnipresent in our environment, and their number keeps growing, it seems highly relevant to investigate the impact of these chemicals on the initiation and progression of tumours, to better understand the high incidence of some hormone-dependent cancers.

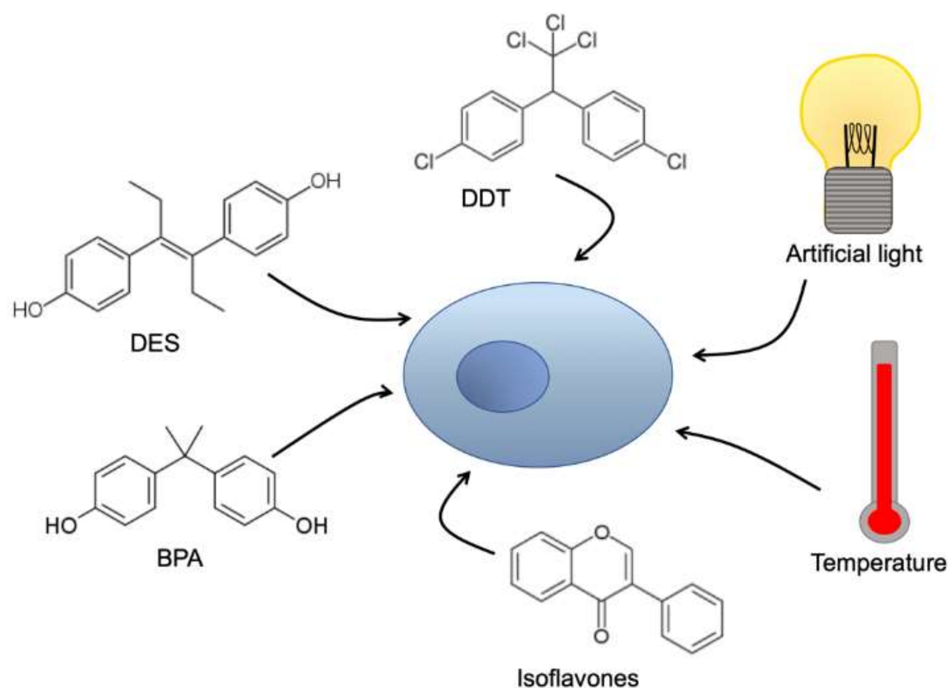

Figure 2.1. Endocrine disruptors in the environment of the cells. This figure depicts the different types of biological, chemical and physical endocrine disruptors affecting cellular physiology.

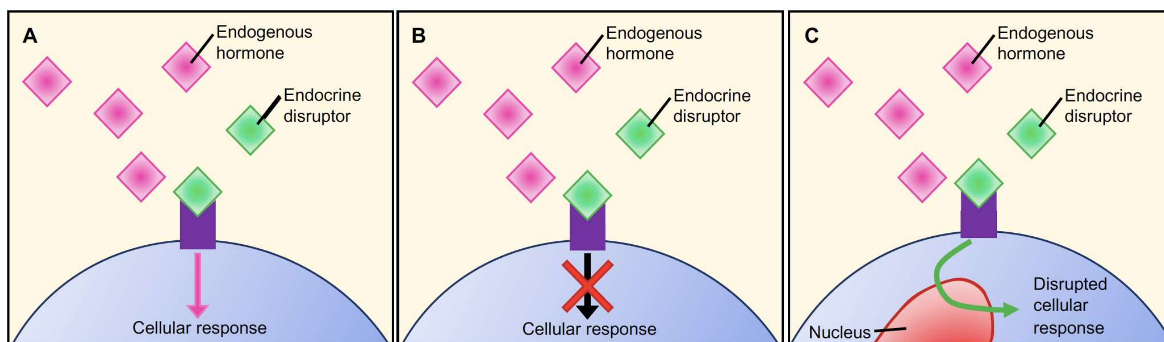

Figure 2.2. Mode of action of endocrine disruptors. Endocrine disruptors can affect signalling pathways, mainly through three mechanisms. **A)** Endocrine disruptors can mimic a naturally-produced hormone, allowing direct activation of hormone receptors. **B)** Endocrine disruptors can bind to the targeted receptor and block the binding of the endogenous hormone. **C)** Endocrine disruptors can affect signalling pathways modulating gene expression, disrupting multiple factors associated with different cellular functions.

Some non-chemical EDs can induce effects on tissues and organs and cause serious consequences. Physical EDs, such as artificial light at night and temperature, disrupt the endocrine system and promote tumour development. Light at night alters the circadian cycle, which is associated with the cell cycle [14, 15], while the temperature potentiates the effects of EDs and alters reproduction and development [16, 17]. Other EDs are naturally found in the environment, such as zearalenone and isoflavones. These natural EDs can also bind to ERs and cause diverse consequences [11, 12, 18].

1.3.1.2 Chemical EDs

In the environment, more than 350 synthetic molecules have been identified as potential EDs because of their capacity to modulate the action of various hormones in the organism [7]. Many have made the news headlines, such as dichlorodiphenyltrichloroethane (DDT), diethylstilbestrol (DES) and, recently, BPs. These molecules bind to ERs with different affinities to modulate their activity. DDT binds to ERs and modulates their signalling pathways, promoting tumour development by altering specific genes associated with cell cycle and inflammation [19, 20]. Nelson *et al*. reported that DES could, among other things,

bind to ERs and cause diverse consequences, such as the emergence of teratogenic and carcinogenic lesions [21]. The most ubiquitous EDs in today's environment are BPs, and their endocrine-disrupting effects deserve extended investigations.

1.3.1.2.1 Bisphenol A (BPA)

BPA is a member of the bisphenol family, as it is composed of two phenol cycles [22]. It is a soluble molecule previously used as a synthetic estrogen [23]. It is still abundantly used in the production of plastics, such as polyesters, epoxy resins and polycarbonate [22]. BPA is found in many daily objects, making this compound ubiquitous in our environment. The principal source of contamination to BPA is food. BPA found in dishes, bottles, and other containers are released in their content when exposed to high temperatures. BPA is also found at measurable concentrations in soil, air and waterways [7, 13]. Once in the organism, BPA is rapidly metabolized and excreted as metabolites. However, a certain proportion stays non-metabolized, which allows its detection in serum and urine in adults and children [22, 23]. BPA detection is mainly performed by high-performance liquid chromatography-tandem mass spectrometry [24, 25]. Persistent concentrations of BPA in the environment and the organisms cause permanent exposure to this ED (**Table 2.1**). BPA has a non-monotonic dose-response activity, which means low and high concentrations have little to no effects, while middle-range exposures have the most significant ones [26]. BPA concentrations found in the organism are usually in the range where this compound is the most effective.

Table 2.1. Bisphenol A concentration in various human biological fluids.

Sample	Concentration	References
Blood	< 0.5–22.3 ng/mL	[27, 28]
Pregnant women serum	< 0.1–154 ng/mL	[29, 30]
Saliva	0.1 ng/mL	[31]
Amniotic fluid	2.80–5.62 ng/mL	
Umbilical cord	< 0.05–52 ng/mL	
Follicular fluid	1–2 ng/mL	
Breast milk	< 0.04–11 ng/mL	[32]
Pregnant women urine	180.1 ng/mL	[33]

BPA binds to multiple receptors, such as ERα and ERβ, AR, estrogen-related receptor γ (ERRγ), G protein-coupled estrogen receptor (GPER) and insulin-like growth factor 1 receptor (IGF-1R) [22]. The multiple binding capacities of BPA allow this molecule to promote tumour development through many signalling pathways, mainly the GPER/epidermal growth factor receptor (EGFR)/extracellular signal-regulated kinase (ERK) pathway. The activation of this pathway promotes the proliferation, growth and migration of cells, such as breast cancer cells, which do not express the classic ERs [22, 34]. Studies on human laryngeal squamous cell carcinoma showed that exposure to BPA binds to GPER, which triggers migration and proliferation via an up-regulation of IL-6 [8]. GPER activates the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway, playing a role in cell growth and proliferation. The activation of this pathway also leads to the inhibition of apoptosis via increased expression of anti-apoptotic factors, such as B-cell lymphoma 2 (Bcl-2), and a decreased expression of pro-apoptotic factors, such as tumour protein 53 (p53) and Bcl-2–associated X protein (Bax) [22]. BPA's activation of the PI3K/Akt pathway has been shown in ovarian cancer cells [13]. GPER binding also activates ERK1/2, promoting cell proliferation. As such, the expression levels of some factors, like EGFR, ERK1/2 and Akt, are increased following exposure to BPA, allowing the cells to transit from a healthy to a cancerous phenotype [35]. Studies have reported that fetal exposure to BPA promotes the abnormal activation of Akt and ERK1/2 in

mammary glands, which increases the risks of developing breast cancer. Finally, GPER promotes the production of matrix metalloproteinases (MMP) -2 and -9, involved in the degradation of collagen in the basal lamina, thus promoting cell migration into the extracellular matrix, and the formation of metastases [22, 36]. MMP-2 production was indeed increased in laryngeal squamous cell carcinoma exposed to BPA [8] as well as lung cancer cells [13], while the expression of both MMP-2 and -9 was increased, through ERRγ, in breast cancer cells exposed to BPA [37]. The EGFR/ERK pathway also seems to be activated through the binding of BPA to ERRγ. Studies on endometrial cancer cells have shown that the activation of ERRγ by BPA can promote cell proliferation via EGFR/ERK pathways [38]. BPA promotes the initiation and progression of a cancerous phenotype through the modulation of many pathways promoting proliferation, migration and cell growth, and the inhibition of apoptosis in affected cells.

1.3.1.2.2 Bisphenol S (BPS)

Since BPA's adverse effects on human health have been identified, many analogs have been produced to replace this problematic compound in daily-used items. One of these molecules is BPS, which has a similar structure to BPA (**Figure 2.3**). BPS is now used as a substitute in industrial plastic products because of its excellent stability [39]. BPS was initially considered safer than BPA, but studies showed that BPS could also act as an ED and affect physiological functions. Eladak *et al.* have shown, in mice models, that exposure to BPS decreased the levels of testosterone, similarly to BPA. BPS also showed anti-androgenic effects and reduced testosterone's secretion in cultured human fetal testes [40]. Just like BPA, BPS accumulates in the organism by ingestion and continuous exposure. A study performed in the United States of America (USA) reported that BPS was detected in 78% of urine samples at concentrations similar to BPA, ranging from 1 to 100 nM [41]. BPS promotes the growth and migration of cultured lung cancer cells via the up-regulation of transforming growth factor-β (TGF-β). Song *et al*. showed that BPS activates Smad2/3, a mediator of the TGF-β pathway. The up-regulation of TGF-β by BPS, via ERα/β, GPER and ERK1/2, also stimulates migration through increased expression of MMP-2 and vimentin, which are migration-associated markers [41]. Studies on cultured neuroendocrine cancer cells by Jia *et al.* demonstrated that BPS triggers the migration and invasion of cancer cells via binding to

ERRα [39]. This association induces an up-regulation of MMP-2 and -9 involved in migration and invasion. It can be noted that BPA and BPS bind to different ERRs: ERRγ for BPA and ERRα for BPS [39]. Therefore, studies seem to demonstrate that BPS has a similar role to BPA in disrupting hormonal signalling, which could promote cancer development.

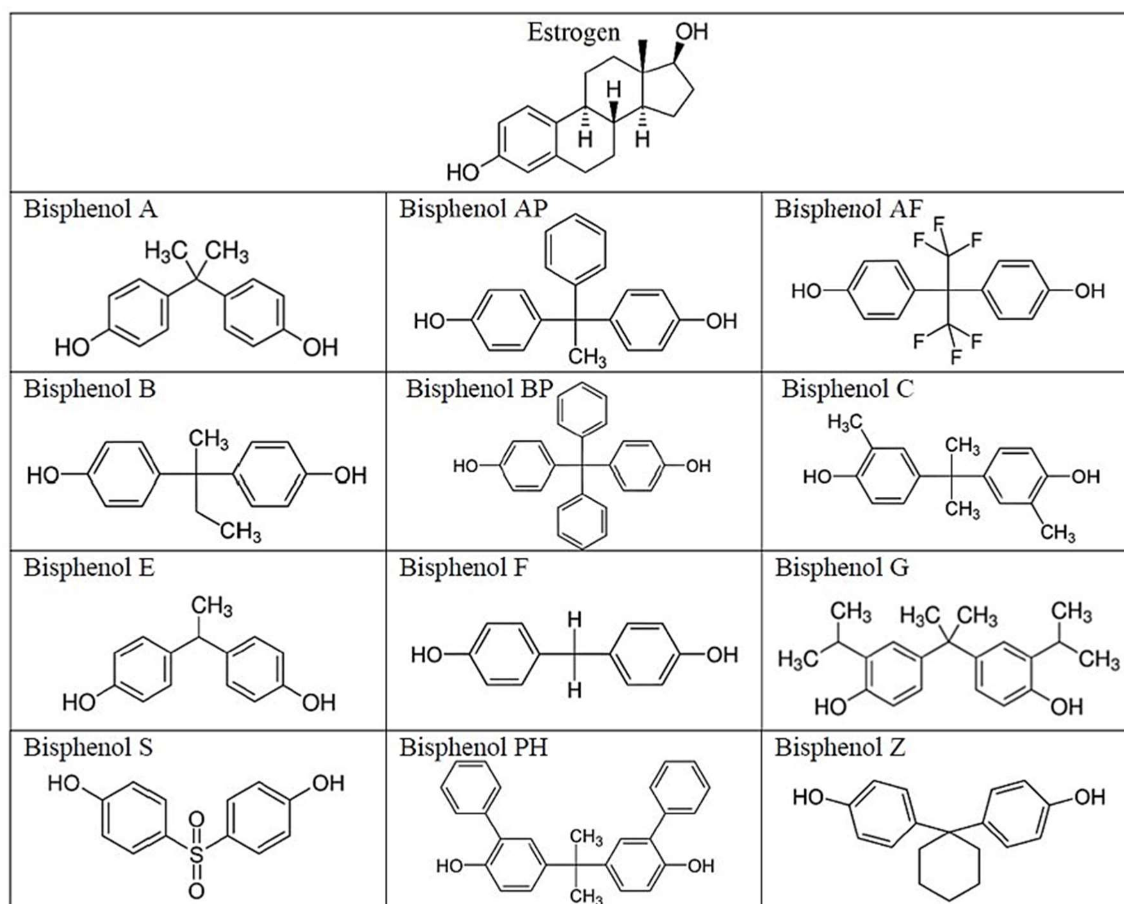

Figure 2.3. The developed formula of members of the bisphenol family compared to estrogen. The various members of the bisphenol family share structural similarities with estrogen. Currently, the most common in our environment are BPA and BPS.

1.3.1.2.3 Bisphenol metabolites

Following ingestion and absorption by the organism, BPA and BPS are usually rapidly metabolized by the liver. BPA has a half-life of approximately 4-5 h in adult humans, but its metabolic rates are lower in fetuses and children [26]. These compounds are converted into different metabolites, but the conjugation with glucuronic acid is one of the most essential

metabolic and detoxification reactions for BPs [42]. A small proportion of BPs is found as sulphate conjugates, the second most common metabolites [42, 43]. 99% of absorbed BPA is metabolized by the liver, while only 41% of BPS is converted into metabolites [44]. These BP metabolites remain active in many organs [45]. These compounds are mainly excreted in the urine and sometimes in the sweat. However, some studies have shown that some organs, such as lungs, liver and kidneys, can be characterized by the presence of a β-glucuronidase enzyme, which can deconjugate BPA and release the original active molecule back in the organism [27].

1.3.2. Bisphenols and non-urological cancers

The effects of BPs on breast cancer, mostly BPA, have been extensively studied in the last decades. BPA could increase proliferation and oxidative stress by binding to ERα and ERβ on human breast cancer cell lines [46]. Also, an exposition to low BPA levels could increase wound closure, therefore cell migration, and invasive behaviour, by a possible increased expression of MMP-2 and -9. BPA could also favour tumour development by altering gene expression following binding to nuclear receptors [13, 34]. BPS could also induce epigenetic and transcriptional modifications, promoting breast cancer development [33, 47]. BPS can increase migration and stimulate cell proliferation through increased cell cycle gene expression [48]. Therefore, BPs can have a significant impact on breast cancer development and progression, and epidemiologic studies should consider its exposition when reporting on large cohorts.

The consequences of BP exposure and the incidence of testicular cancer have not been demonstrated yet. It is known that the incidence of testicular dysgenesis syndrome (TDS) has dramatically increased in the last decades [49]. TDS includes conditions characterized by signs, symptoms or disorders, such as hypospadias, cryptorchidism (undescended testis) and testicular cancer. The increased incidence of TDS could be linked to chronic exposure to EDs, such as BPs, capable of altering hormone signalling pathways [49]. Precocious exposure to BPA can cause over-expression of specific hormone receptors in the testis, which increases the sensitivity to endogenous and artificial hormonal compounds [50]. By altering

the hormone balance in the organism, precocious exposure to BPs could increase TDS risks and the development of testicular cancer.

Several other organs could be chronically affected by BPs. Evidence for cancer initiation and progression following BP exposure such as colorectal [51], oral and oropharyngeal [52], liver [53], pancreas and thyroid cancers [54] have been described. This review will focus on two types of urological cancers that share standard carcinogenic processes: PCa and BCa.

1.3.3. Bisphenols and urological cancers

PCa and BCa may share a common carcinogenic process or a susceptibility to develop both cancers. Studies have reported that the frequency of patients with BCa developing PCa is as high as 70% [55]. Even though the frequency of patients with PCa developing BCa is about 3.4%, some studies have reported that an association can be made between these two cancers and N-acetyltransferase polymorphisms linked with altered DNA repair [55, 56]. The literature shows growing evidence of the impact of BPs on PCa initiation and progression (reviewed below). However, there is no direct evidence indicating that BPs affect BCa. This review discusses different elements that suggest BPs could indeed affect BCa initiation and progression. These links suggest that more research is needed to confirm or infirm this hypothesis.

1.3.3.1 Prostate cancer

PCa is one of the most frequent cancers diagnosed in men, with 174,650 new cases and 31,620 deaths from PCa in the United States for 2019. PCa can be cured if diagnosed at an early stage. Indeed, more than 2.9 million men in the United States diagnosed with PCa are still alive in 2019. However, PCa represents the second leading cause of cancer death in the USA behind lung cancer and is the most commonly diagnosed lethal cancer in men [5]. Among the different risk factors, infection, inflammation, age, diet, and genetics are the most associated with PCa. Recent studies brought interest to the excessive secretion of androgen and other male sex hormones and their impact on PCa. The growing ubiquity of EDs such as

BPA as a new source of risk factor source has been an emerging field to determine its effect on PCa.

Since estrogens can initiate PCa and drive its progression in both humans and rodent models [57, 58], the hormonal sensitivity is a characteristic of the cell lines used to create cancer models. As such, two types of PCa cells are used, namely cells highly sensitive to androgen (LuCap 23.1 and LNCaP), where the downregulation of AR expression can decrease cell growth by reducing AR-mediated transcription, and cells with reduced androgen sensitivity and increased metastatic capability (C4-2 and IGR-CaP1) [59]. It is, therefore, believed that androgens could impact PCa prevalence. As previously discussed, the similarity between androgens and BPs suggests that these latter could play a role in PCa initiation. Indeed, BPA has even been shown to act as a weak estrogen receptor agonist in the human brain and is nowadays considered an androgen disruptor [60]. BPA was, for the first time, reported having an estrogenic activity in 1936 [61].

BPA is a weak estrogen compared to estradiol because of its low binding affinity for classical nuclear ERs. However, BPA affinity is strong for the ERRγ and the GPER. Furthermore, its potency for non-nuclear ERs associated with rapid-response enzyme cascades is equal to estradiol [62]. Male sexual dysfunction and alterations in serum hormone levels have been associated with exposure to BPA in Chinese adult men [62]. Furthermore, a significantly higher urinary BPA-glucuronide level has been found in biopsy-confirmed PCa patients compared to those with no cancer diagnosis [63]. Further, *in vitro* and *in vivo* studies identified molecular pathways, through the stimulation of AR and ER gene expression, used by BPA to mediate its effects on the prostate [26]. Burton *et al.* showed that BPA and estradiol interfere with the expression of histone deacetylase SIRT1 and histone methyltransferase SET8, causing gene expression modifications in PCa cells [64].

Depending on the developing stage of the fetus [60], the exposition to BPA during development has shown to induce abnormalities to the male reproductive system, including the prostate [65], and changes in gene expression within the prostate in mice [66]. Prenatal exposure to BPA has been linked to changes in key genes associated with PCa in rats [67]. Therefore, the function of normal physiologic estrogen can be disrupted by exposure to

xenoestrogen mimetics such as BPA [68]. An alteration of physiology can be expected from EDs because of the interference of their hormone actions and homeostasis disruption [9]. Because of the equilibrium between self-renewal and differentiation, prostate stem cells play a significant role in PCa. The presence of EDs may modify this equilibrium [69]. Recent studies have also shown that BPA can target human prostate stem and progenitor cells and reprogram them even after a low-dose exposure [70]. The influence of BPA on embryonic and adult prostate stem cells has been deeply studied (proliferation, gene expression) and already reviewed [26].

1.3.3.1.1 Factors impacting prostate cancer

In the healthy prostate tissue, growth and differentiation of epithelial cells are known to be regulated, in part, by androgens. Indeed, healthy prostate is characterized by the presence of ERα, suggesting that estrogens play a direct role in prostatic epithelial cells [71]. Krishnan *et al.* showed that low levels of BPA significantly increased the proliferation of ER-positive cells [72]. Recent studies even demonstrated the impact of ERs in the induction of PCa [73]. ERα was found to be up-regulated and to mediate the carcinogenic outcome of estradiol. In contrast, ERβ, the most prevalent ERs in the human prostate, displays tumour suppressor activities. Its expression is lost in castration-resistant cancers, which will be further detailed in this review.

At an early stage of PCa, cell growth and development are regulated by AR and ERs [74]. Prostatic adenocarcinoma occurred in every experimental rat after treatment with 17β-estradiol or with DES plus testosterone for 44 and 59 weeks, respectively [75]. Furthermore, Ricke *et al.* demonstrated that prostates in mice treated with 17β-estradiol and testosterone remained free of pathology in ERα-knockout mice. Simultaneously, carcinogenesis occurred in wild-type and ERβ-knockout mice, suggesting that effective prevention of carcinogenesis will require antagonism of ERα, but not ERβ. According to Ricke *et al.* by homology to androgens, ingested BPs could attach to ERα and promote prostate carcinogenesis [76]. Due to the estrogenic ability of BPs and its capacity to bind on AR, BPs could contribute to PCa initiation and progression in the early stages [74]. Whether BPA is an estrogenic chemical or

not is not to debate anymore. *In vitro* and *in vivo* studies showed BPA's potency to act, even at low doses, as an estrogen mimetic by inducing endocrine disruption [77].

The PCa metastatic progression is the leading cause of mortality and morbidity. It is associated with the cancer-associated cell migration protein tetraspanin 1 (TSPAN1), which is induced by androgens, and significantly up-regulated in PCa. Indeed, the up-regulation of TSPAN1 in both DU145 and PC3 cells (which are not detectably hormone-sensitive) significantly increased cell migration, suggesting that TSPAN1 is an androgen-driven contributor to cell survival and migration in PCa [78]. The homology between androgens and BPs suggests they could activate and up-regulate the TSPAN1 receptor, promoting metastatic progression. Also, BPA has been shown to significantly stimulate cell migration and invasiveness of LNCaP cells through the upregulation of the Ca^{2+} ion channel gene [79]. The hypothesis of a role for BPA in the onset and progression of PCa was supported by a prospective population-based study describing positive associations between higher BPA concentrations and higher expression of *ESR2* (the gene encoding estrogen receptor 2 or ERβ) and *ESRRA* (the gene encoding ERRα) [80]. Since androgens and estrogens play a central role in the onset of PCa, BPA could be responsible for promoting PCa through AR or ERs. By activating the nuclear receptor-mediated pathway, a role for BPA as a xenoestrogen can strongly be suspected in men: could it also interfere with PCa treatments?

1.3.3.1.2 Potential impact of BPA on prostate cancer treatments

Radical prostatectomy or radiation-based therapies are the primary treatments for patients with organ-confined PCa [81]. However, 25 to 40 % of patients will present a rise in prostate-specific antigen (PSA), an androgen-regulated biomarker of recurrent prostate tumour growth and metastatic progression [81]. Development and maintenance of normal and malignant prostate tissues being regulated by the androgen-dependent gene pathways, androgen-deprivation therapy (ADT), namely the suppression of testicular testosterone production, has therefore been set to treat recurrent and metastatic PCa [81-83]. In the presence of BP exposure, we can hypothesize that BPs could challenge the ADT's effectiveness by acting like endogenous androgens. Despite the lack of studies in the literature, castrate-resistant prostate cancer (CRPC) could also be explained by low

concentrations of BPs in the serum, therefore mimicking androgens and making castration inefficient. Castration dramatically decreases the concentration of androgens, but BPs could replace the role of endogenous androgens by their chemical nature.

Studies aimed to determine the mechanisms contributing to the reactivation of the AR in PCa progression. Splicing events or mutations in the ligand-binding domain of the AR induce an increase of modified receptors, which allows the activation by other molecules than androgen-like steroid hormones or anti-androgens [84]. By structural homology, it is possible that BPs, like BPA, could bind to modified AR and cause its reactivation. The amplification of the *AR* gene can also explain the reactivation. It has been reported that this *AR* gene amplification characterizes 30 % of tumour samples and is frequently found with an increased AR stabilization [85]. This means that more AR is produced and, therefore, fewer androgens are needed to activate AR and its pathways, promoting CRPC. In the presence of ADT, PCa cells can up-regulate the necessary enzymes to synthesize their own androgens *de novo* [81]. To comfort this hypothesis, Mostaghel *et al.* observed that intraprostatic levels of testosterone in CRPC were high even in the presence of low circulating androgen concentrations due to ADT [86]. Thus, the PCa cells microenvironment gathers enough autocrine and paracrine supply of androgens to reactivate the AR pathway and support CRPC [87].

The centrosome is an emerging therapeutic target in CRPC [23]. One or two centrosomes generally characterize normal cells. However, Tarapore *et al.* showed that BPA treatment of normal or cancerous prostate cell lines increased the frequency of cells having three or more centrosomes [63], which is commonly observed in human tumours, and contributes to chromosomal instability [88]. Correlation between PCa and BPA are, therefore, strengthened by these findings. Furthermore, BPA is known to induce proliferation in LNCaP through a muted AR, known as AR-T877A receptor [89], facilitating the transition to a CRPC. By changing the shape and the size of the receptor by modifying its amino acids, the loss of ligand specificity is recurrent in advanced PCa [90]. Therefore, the receptor can be activated via many different compounds (including BPA), promoting PCa growth [91]. Pieces of evidence indicate that the absence of androgen can be circumvented with BPA by activating common tumour-derived AR mutants [23]. Thus, it can be proposed that the presence of BPs

could contribute to the autocrine and paracrine supply of androgen to reactivate these receptors and promote CRPC.

1.3.3.2 Bladder cancer

BCa is the fourth most common cancer diagnosed in men and the eleventh most common cancer diagnosed in women, representing 80,470 new cases and 17,670 deaths in the USA for 2019. Relatively older adults are affected by this cancer, as 9 out of 10 patients are aged over 55 years old, and the average age at the time of diagnosis is 73. The odds of developing this type of cancer are about 1 in 27 for men and 1 in 89 for women [5]. This gender difference may be due to different risk factors and the regulation of sex steroid hormones [92]. However, even after controlling carcinogenic factors such as smoking or exposure to industrial work-related chemicals, it remains predominant in men. Consequently, intrinsic factors may play a critical role in BCa [93].

1.3.3.2.1 Factors impacting bladder cancer

The role of the various sex-hormone receptors, such as ERα, ERβ and AR, remains little studied in BCa. Estrogen-like metabolites have been identified in *Schistosoma haematobium*-infected patients' urine in which ERα activation promotes proliferation in BCa cells [94]. ERα seems to inhibit the BCa invasion by decreasing circular RNA levels, such as circ_0023,642, and increasing miR-490-5p, resulting in decreased EGFR expression [95]. ERβ expression seems to increase with tumour stage progression [96] and was found to be greater in high-grade and muscle-invasive tumours [97]. ERβ promotes BCa growth and invasion through upregulation of miR-92a and then decrease in DAB2IP tumour suppressor expression [98]. The activation of ERβ and AR correlates with the inactivation of FOXO1, a tumour suppressor likely turned off in BCa, which induces urothelial carcinogenesis and cancer growth [99]. ERβ activation also seems to play a role in the control of apoptosis to protect BCa cells. ERβ signalling induces cisplatin resistance in BCa in part through the expression and activity of β-catenin [100]. Cancer-associated fibroblasts promote cisplatin resistance in BCa cells by increasing the anti-apoptotic protein Bcl-2 through ERβ signalling [101].

Preclinical studies suggested the involvement of molecules and pathways regulated by AR signals in the development of urothelial cancer [93]. AR expression remains controversial in urothelial tumours. Studies indicate that AR appears to be reduced in urothelial tumours compared to native tissues, with an expression of about 13-78% for BCa or upper urinary tract urothelial tumours, and 58-86% for healthy tissues [93, 102]. The AR expression decreased with an increasing pathological stage [103]. The AR expression is associated with better survival, whereas the ERβ expression is associated with worse recurrence-free and progression-free survival in non-muscle-invasive BCa [104]. As suggested by Ide *et al.* a correlation between high expression of the AR and a lower risk of BCa recurrence might exist [102]. However, Mizushima *et al.* propose AR positivity as a predictor of tumour progression [93]. Nevertheless, induction of BCa with the chemical carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine ultimately failed in AR-knockout mice, suggesting a critical role of AR in the induction of tumours [105]. Androgens, like testosterone, have also been shown to increase the incidence of tumours in female rats [106]. The AR contribution to BCa progression has been assessed by androgen deprivation and AR knockout *in vitro*: Indeed, apoptosis has been increased, cancerous cell proliferation *in vitro* and xenograft tumour growth *in vivo* suppressed, thereby involving AR in BCa progression [105]. The AR modulates signalling pathways, e.g., pathways involving β-catenin or cyclin D to promote the carcinogenesis and aggressive biological behaviour [107-109]. As the AR is contributing to BCa progression, it can be hypothesized that, by homology with androgens, BPs could activate this receptor and promote tumour growth.

At a genetic level, AR responsiveness could be explained by the presence of the androgen response element (ARE) in the promoter region of the *PSCA* (prostate stem cell antigen) gene. If this region loses AR responsiveness (by over-activation, for example), an androgen-independent mechanism could develop and increase the metastatic potential [110]. By homology with PCa, it can be hypothesized that BPA, due to its presence in body fluids, could play a leading role in the onset of BCa and its progression through the AR. By over-activation, AR responsiveness could be lost, promoting the metastatic potential. However, the current lack of data prevents the establishment of a link between BPA and BCa.

A study has reported that AR-mediated signals modulate the activity of enzymes involved in the metabolism of bladder carcinogens, including cytochromes P450, a superfamily of detoxifying enzymes [93]. Besides metabolism, androgens positively impact cell proliferation by increasing the growth of AR-positive BCa cells, but not those of AR-negative BCa [93].

1.3.3.2.2 Impact on cell proliferation

The utilization of AR antagonists or AR knockdown induces impaired cell proliferation of BCa cells cultured with androgens. Indeed, it has been shown that androgens directly or indirectly regulate molecules and pathways known to play a central role in BCa cell proliferation such as β-catenin, the cluster of differentiation 24 (CD24), erythroblast transformation specific like-1 protein (ELK1) and cyclins [93]. It is known that β-catenin participates in the Wnt signalling pathway, which is implicated in cell proliferation and migration. Indeed, activation of the Wnt/β-catenin pathway has been linked to higher tumour grades and worse patient outcomes [93]. Co-immunoprecipitation of AR and β-catenin in BCa cell lines suggests a close interaction between these components and may implicate downstream Wnt signalling components [107]. CD24 is a cell adhesion glycoprotein and a cancer stem marker implicated in tumour and metastasis. Its high expression in BCa has been linked with poor patient outcomes. However, CD24 knockdown in AR-positive BCa cells induced a growth reduction [111] and prevented metastasis development in animal models [111]. Finally, cell growth of AR-positive BCa cell lines is enhanced in the presence of androgen treatment through the *GON4L* gene (regulating the expression of CD24), which drives cancer growth by the Yin Yang 1 (YY1)-AR-CD24 axis [112]. Cyclins, especially the cell-cycle progression regulator cyclin D1, are known to modulate BCa cell proliferation. Studies demonstrated that AR knockdown or androgen deprivation in BCa cells induced down-regulation of cyclin D1. Therefore, the presence of androgen may be linked to the expression of cyclin D1, which over-expression enhanced BCa outgrowth [113]. The transcription activator ELK1 is known to modulate cell-cycle control, apoptosis and cell proliferation. Its expression has been significantly correlated with AR in BCa. Indeed, the proliferation of BCa cell lines could be triggered following the activation of ELK1 with the

use of androgens [114]. Also, inactivation of ELK1 in BCa cells using α1A-adrenergic blocker induced growth inhibition [114].

Despite the lack of information in the literature, it can be hypothesized that as BPA shares structural homology with androgens, its presence in human blood could impact cell proliferation by activating the AR and therefore modulate the Wnt-1 pathway. It could also impact tumour growth through the YY1-AR axis. Through binding to AR, BPA could up-regulate cyclin D1 and thus enhance BCa outgrowth. Following binding to androgen-activated ELK1 receptors, BPA could also induce cell proliferation and reduce apoptosis through ELK1 activation. Experiments could be performed to examine the impact of BPs and its role as an androgen-mimicking molecule on the Wnt-1 pathway, YY1-androgen receptor, cyclin D1 and ELK1 receptors.

1.3.3.2.3 Migration and invasion

Migration and invasion are tightly correlated with cell proliferation to allow proper development and cell spreading. Various studies reported a higher migration capacity and invasion of BCa cell lines through the AR pathway [114-116]. Studies have shown that AR knockdown or anti-androgen treatments inhibit androgen-mediated cell migration and invasion by modulating molecules and pathways known for being implicated in BCa. Examples of these are, as seen before, β-catenin, CD24 and ELK1, as proliferation is closely linked to migration and invasion, but also MMPs [93, 116]. Indeed, in BCa cells, MMP-2 and MMP-9 are over-expressed when the AR is up-regulated and, on the contrary, the expression of these MMPs are down-regulated following AR knockdown [115, 117]. MMP-10 [118] and MMP-13 [119] were also over-expressed by the activation of the AR in BCa cells. As for cell proliferation, we can suggest that, by homology with androgens, BPA could promote over-expression of MMPs and cell migration through tissues, which could negatively impact patients' outcomes. BPA could use the same pathway used to trigger PCa but in the BCa cells.

1.3.3.3 Potential impact of BPA on bladder cancer treatments

The incidence of BCa in men with PCa treated by ADT was significantly lower than those treated by surgery alone [120]. Furthermore, the treatment of men with both PCa and BCa with ADT actively prevented the recurrence of BCa compared to patients without hormonal therapy [121]. Raloxifene and tamoxifen are hormonal treatments acting via ER mechanisms that were shown to inhibit urothelial carcinoma proliferation *in vitro* and *in vivo* [92, 122-124]. EGF has been shown to improve BCa cell growth and migration through the AR, even under a low amount of androgen level. Indeed, EGF could potentiate AR transactivation through EGFR by activating PI3K/AKT and mitogen-activated protein kinase (MAPK) pathways, even under a castration androgen level [125]. It can, therefore, be a potential target to improve therapeutic outcomes. These *in vitro* and *in vivo* results suggest the combination of both treatments to improve patient outcomes. In the presence of BPs, it could be of interest to evaluate these AR-related treatments to determine if BPs could mimic androgens and, therefore, negatively impact BCa patients' outcomes.

Recent preclinical studies suggested that the AR activity could negatively impact the success of standard treatment. First, chemoresistance to cisplatin and doxorubicin have been positively correlated to AR activity in BCa cell lines. *In vitro* studies confirmed that AR-positive cell lines were more resistant to cisplatin than AR knockdown cells or AR negative controls [126]. Furthermore, AR expression was considerably higher in the doxorubicin- or cisplatin-resistant sublines created by long-term cell culture with gradually increasing concentrations of cisplatin/doxorubicin, compared to control cells [113, 126]. It could be interesting to evaluate the chemoresistance to cisplatin and doxorubicin in the presence of BPs. These components may, by over-activation of AR, induce resistance of cancerous cells.

Numerous treatments exist to combat PCa and BCa, which present strengths and weaknesses. A substantial proportion of patients have been successfully treated and cured, yet many patients must face recurrence, even after radical treatment. The AR seem to be an innovative target, as it is involved in cell proliferation, migration and invasion. However, we hypothesize that the presence of analogs such as BPA or BPS could, due to their structural homology with estrogen, bind AR and directly impact cancer onset and patients' outcomes. As PCa and BCa

are hormone-dependent, BPs could reduce standard treatment efficacy. In the case of PCa, for example, chemical castration efficacy could be impacted by BPs acting as endogenous estrogen-like molecules, reactivating the AR pathway and support CRPC. In this respect, the impact of BPs should be evaluated to determine its role in PCa and BCa progression. Although BPA's role in triggering PCa is an emerging domain, the current lack of data makes this role somewhat controversial, especially for BCa. It may be due to the difficulty in assessing exposure during susceptible life stages, the short *in vivo* half-life and the constant growing list of chemicals similar to BPA used on the market to replace it.

1.3.4. Future directions

EDs have made the news headlines for several years now. Regulatory agencies from countries worldwide, such as Canada, France, China and Brazil, have adopted laws to restrict the use of BPA (**Figure 2.4**). For example, Canada (https://www.canada.ca/en/health-canada/services/home-garden-safety/bisphenol-bpa.html), the USA (https://www.fda.gov/food/food-additives-petitions/bisphenol-bpa-use-food-contact-application) and the European Union (https://ec.europa.eu/commission/presscorner/detail/en/IP_11_664) have banned the use of BPA for baby bottles and sippy cups. The EU has also restricted BPA use in thermal papers such as cash receipts (https://chemicalwatch.com/88020/restriction-of-bpa-in-thermal-paper-enters-into-force-in-eu). More restrictions should be put in place, such as banning BPA in all plastic bottles and metal cans coating.

Nonetheless, and despite growing evidence in the literature that BPs could be involved in various pathologies, including cancers, the ban of BPs is rarely exerted to BPA alternatives and often only applies to BPA itself. Pollutants like plastics are ubiquitous in our environment, and their long lifespan causes a continuous accumulation, mainly because of the exponential growth of plastic production. The constant presence of EDs in our environment during the last decades could affect future generations. Extensive research is needed to establish the putative roles of BPs on public health and understand the underlying mechanisms better, causing their toxicity [127, 128]. It will allow the establishment of better strategies to avoid the consequences associated with continuous exposure to these pollutants as much as possible.

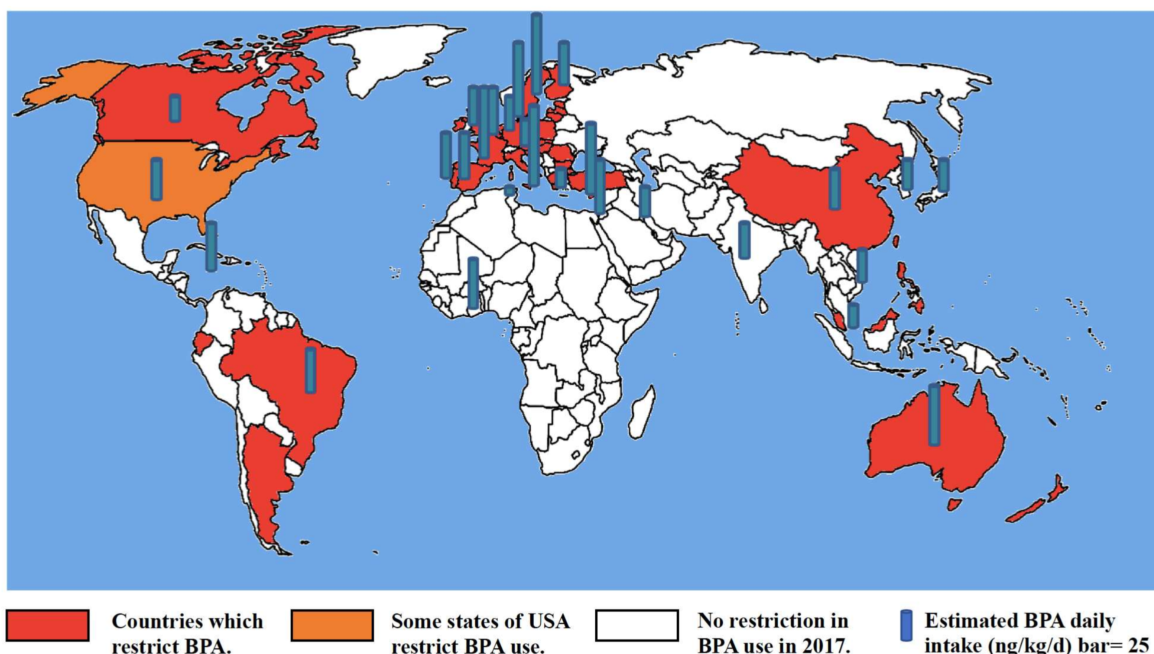

Figure 2.4. World regulatory of BPA use in 2017 versus estimated BPA intake. Countries that restrict BPA usage are represented in red. The USA, where BPA is only restricted in some states, is represented in orange. Other countries are represented in white. BPA daily intake is represented by blue bars. Data are extracted from [129].

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Chapitre 2 : Le bisphénol A et le bisphénol S altèrent le métabolisme énergétique et le comportement des cellules urothéliales saines et cancéreuses de vessie

2.1 Résumé

Le bisphénol A (BPA) et le bisphénol S (BPS) sont des perturbateurs endocriniens utilisés dans la fabrication des plastiques. Ces composés sont respectivement retrouvés dans >90 % et 78 % des échantillons d'urine chez l'humain à des concentrations similaires. L'urothélium vésical est donc exposé de manière chronique aux BP. L'exposition au BPA et au BPS est associée à la progression tumorale via leur liaison à certains récepteurs hormonaux. La présence de ces récepteurs sur l'urothélium vésical et leur rôle dans l'initiation et la progression du cancer de la vessie suggèrent que les BP pourraient avoir un impact sur le développement de ce cancer. Nos résultats démontrent que l'exposition chronique aux BP diminue la bioénergétique et les propriétés des cellules urothéliales, tout en les augmentant pour les cellules cancéreuses de vessie. Ces résultats suggèrent que l'exposition au BPA et au BPS pourrait favoriser le développement du cancer de la vessie.

2.2 Abstract

Bisphenol A (BPA) and bisphenol S (BPS) are used in the production of plastics. These endocrine disruptors can be released into the environment and food, resulting in the continuous exposure of humans to bisphenols (BPs). The bladder urothelium is chronically exposed to BPA and BPS due to their presence in human urine samples. BPA and BPS exposure has been linked to cancer progression, especially for hormone-dependent cancers. However, the bladder is not recognized as a hormone-dependent tissue. Still, the presence of hormone receptors on the urothelium and their role in bladder cancer initiation and progression suggest that BPs could impact bladder cancer development. The effects of chronic exposure to BPA and BPS for 72 h on the bioenergetics (glycolysis and mitochondrial respiration), proliferation and migration of normal urothelial cells and noninvasive and invasive bladder cancer cells were evaluated. The results demonstrate that chronic exposure to BPs decreased urothelial cells' energy metabolism and properties while increasing them for bladder cancer cells. These findings suggest that exposure to BPA and BPS could promote bladder cancer development with a potential clinical impact on bladder cancer progression. Further studies using 3D models would help to understand the clinical consequences of this exposure.

2.3 Article

Bisphenols A and S alter the bioenergetics and behaviours of normal urothelial and bladder cancer cells

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Simple Summary: This research brings new knowledge on the potential roles of bisphenol A and bisphenol S on bladder cancer progression. By assessing the impact of bisphenols A and S on normal urothelial cells and non-invasive and invasive bladder cancer cells, this study aimed to demonstrate that these endocrine-disrupting chemicals could promote bladder cancer progression through the alteration of the bioenergetics and behaviours of healthy and cancerous bladder cells. These results could provide a better understanding of the pathophysiology of bladder cancer and its hormone-sensitive characteristics. Furthermore, this study suggests that bisphenols A and S could affect bladder cancer recurrence, progression and patient prognosis.

2.3.1. Introduction

In the last decades, hormone-dependent cancers, such as breast [1] and prostate cancers [2], have increased in industrialized countries. Among several causes, part of this rise could be associated with the growing number of endocrine disruptors found in the environment [3]. More than 350 synthetic molecules in the environment, including bisphenols (BPs), are considered endocrine disruptors because of their capacity to modulate the action or the metabolism of various hormones in the organism [3].

The bisphenol family comprises many molecules, such as bisphenol A (BPA) and bisphenol S (BPS). BPA is used to produce plastics, such as polycarbonate and epoxy resins [4]. It is found in various daily objects (e.g., water bottles and food containers) [5], making this compound ubiquitous in the environment [6]. Exposure to BPA has been associated with cancer development, especially in hormone-dependent cancers such as breast [7] and prostate cancers [8]. Recently, BPS has been used as a safer alternative to BPA in plastic production because of its excellent stability [9]. However, BPS has also been linked with cancer progression [7, 10]. BPA is found at measurable concentrations in the urine of >90% of the population [10], whereas BPS is detectable in 78% of urine samples at comparable concentrations to BPA [11]. BPA and BPS are found at similar concentrations in urine, ranging from 1 to 100 nM [11]. The ubiquitous presence of BPA and BPS in the urine results in chronic exposure to the urinary tract, particularly the bladder, where urine can be stored for many hours [12].

The multiple binding capacities of BPA and BPS allow the compounds to alter different signalling pathways associated with cell migration, proliferation and invasion [10, 13]. In addition, studies have shown that the bladder's urothelial cells (UCs) express cell receptors targeted by BPA and BPS, such as estrogen receptors (ERs) α and β (ERα and ERβ), the androgen receptor (AR) and the G protein-coupled estrogen receptor (GPER) [14, 15]. Although the bladder is not recognized as a hormone-sensitive tissue, studies have shown the role of these sex steroid receptors in bladder cancer initiation and progression. As such, the activation of ERα could promote the proliferation of bladder cancer cells [16], whereas ERβ and AR could promote bladder cancer growth and invasion via the alteration of tumour

suppressor gene expression [17, 18]. Therefore, the pro-tumorigenic tendencies of BPA and BPS, their binding capacities to cell receptors and the presence of these key receptors in the bladder urothelium could suggest a potential role for BPs in bladder cancer development [19].

It was previously demonstrated that the energy metabolism of healthy bladder fibroblasts decreased after chronic exposure to physiological concentrations of BPA. In contrast, BPA-exposed cancer-associated fibroblasts (CAFs) displayed an increased glycolytic metabolism, leading to extracellular acidification [20]. This enhanced acidification can lead to the inhibition of immune cells, such as monocytes, as well as reorganization of the extracellular matrix [21, 22], thus potentially promoting bladder cancer progression [20]. The hypothesis of this study was that BPA and BPS would impact the energy metabolism and properties of normal urothelial cells and bladder cancer cells, i.e., migration, proliferation and expression of cell markers of invasive potential, which could promote bladder cancer initiation and progression.

2.3.2. Materials and Methods

2.3.2.1. Cell lines

All procedures involving patients were conducted according to the Helsinki Declaration and were approved by the local Research Ethical Committee. Each specimen was obtained with the donor's consent for tissue harvesting, and all experimental procedures were performed according to the CHU de Québec-Université Laval guidelines. Normal urothelial cells (UCs) were extracted from healthy human urological tissue biopsies and cultured as previously described [23, 24]. The UCs were isolated from two healthy paediatric volunteers undergoing surgery for a benign condition (UC1 and UC2) and used as non-transformed primary cell lines.

UCs, RT4 non-invasive bladder cancer cells (ATCC HTB-2) and T24 invasive bladder cancer cells (ATCC HTB-4) were maintained in culture media composed of a 3:1 mix of Dulbecco–Vogt modification of Eagle's medium (DMEM) (Invitrogen, Burlington, ON, Canada) and

Ham F12 medium (Invitrogen) supplemented with 5% fetal bovine serum clone II (Hyclone, GE Healthcare Life Science, Wauwatosa, WI, USA), 24.3 μg/mL adenine (Corning, Tewksbury, MA, USA), 5 μg/mL insulin (Sigma-Aldrich, Oakville, ON, Canada), 0.212 μg/mL isoproterenol (Sandoz, Boucherville, QC, Canada), 0.4 mg/mL hydrocortisone (Calbiochem, San Diego, CA, USA), 10 ng/mL epidermal growth factor (Austral Biologicals, San Ramon, CA, USA), 100 U/mL penicillin (Sigma-Aldrich) and 25 mg/mL gentamicin (Schering-Plough Canada Inc./Merck, Scarborough, ON, Canada), and incubated at 37 °C with 8% CO_2. Media were changed three times per week.

2.3.2.2. Seahorse energy metabolism measurements

UCs, RT4 and T24 cells were plated in 96-well Seahorse XF cell culture plates (Agilent/Seahorse Bioscience, Chicopee, MA, USA) and exposed or not to 10^{-8} M BPA (Millipore Sigma, Oakville, ON, Canada) or 10^{-8} M BPS (Millipore Sigma) for 72 h before measurements. Media were changed every day. Seahorse XFe96 sensor cartridge plates (Agilent/Seahorse Bioscience) were hydrated the day before the analysis with the XF Calibrant (Agilent/Seahorse Bioscience) and incubated overnight at 37 °C without CO_2. Before the bioenergetics measurements, cells were washed and incubated for one hour with Glyco Stress media or Mito Stress media. Glyco Stress media contained XF Base Medium (minimal DMEM) (Agilent/Seahorse Bioscience) supplemented with 2 mM L-glutamine (Wisent Bioproducts Inc., Saint-Jean-Baptiste, QC, Canada). Mito Stress media consisted of XF Base Medium supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate (Wisent Bioproducts Inc.) and 10 mM D-(+)-glucose (Millipore Sigma). The extracellular acidification rate (ECAR), representative of glycolytic metabolism, and the oxygen consumption rate (OCR), representative of mitochondrial respiration, were determined using the XFe Extracellular Flux Analyzer (Agilent/Seahorse Bioscience) [20, 25].

The glycolytic metabolism was established by the sequential injection of 10 mM D-(+)-glucose (Millipore Sigma), 1.5 μM of the ATP synthase inhibitor oligomycin (Cayman Chemical, Ann Arbor, MI, USA) to inhibit mitochondrial respiration and force the cells to maximize their glycolytic capacity, and 50 mM 2-deoxy-D-glucose (2-DG) (Alfa Aesar, Ward Hill, MA, USA), a competitive inhibitor of the first step of glycolysis.

The mitochondrial respiration was determined by the sequential injection of 1.5 µM of the ATP synthase inhibitor oligomycin (Cayman Chemical), 0.5 µM of the mitochondrial uncoupler trifluoromethoxy carbonylcyanide phenylhydrazone (FCCP) (Cayman Chemical) and a combination of 0.5 µM of the mitochondrial complex I inhibitor rotenone (MP Biomedicals, Santa Ana, CA, USA) and 0.5 µM of the mitochondrial complex III inhibitor antimycin A (Millipore Sigma). The concentrations indicated for each injection represent the final concentrations in the wells. At least three measurement cycles (3 min of mixing + 3 min of measuring) were completed before and after each injection.

The OCR and ECAR were calculated using Wave software v2.6 (Agilent/Seahorse Bioscience). Following the manufacturer's instructions, energy metabolism was normalized according to the number of cells using a CyQuant Cell proliferation assay kit (Invitrogen). The fluorescence of each well was measured at 485 nm/535 nm for 0.1 s using the Victor2 1420 MultiLabel Counter plate reader (Perkin Elmer Life Sciences, Waltham, MA, USA) and Wallac 1420 software (Perkin Elmer). The normalization values were calculated from the fluorescence measurements with Microsoft Excel software (Microsoft, Redmond,WA, USA) and applied to the metabolic values. Metabolic values were presented as percentages with 100% established using the first three measurements. Therefore, the baseline was established before the injection of glucose or oligomycin (see Figures S1–S4). Each experiment included at least three replicates per condition ($n \geq 3$), and each experiment was repeated at least three times ($N \geq 3$).

2.3.2.3. Proliferation

On day 0, UCs, RT4 and T24 cells were seeded in 24-well culture plates at 60,000 cells/well density. Cells were incubated for two hours at 37 °C with 8% CO_2 to allow cell adhesion and then treated or not with 10^{-8} M BPA or BPS. The medium, supplemented or not with BPs, was changed daily for three days. On days 1 to 3, cells from three wells were collected with trypsin, centrifuged at 300 g for 10 min, resuspended in 10 mL ISOTON II diluent (Beckman Coulter, Mississauga, ON, Canada) and counted separately using a Z2 Coulter Particle Count and Size Analyzer (Beckman Coulter) [26]. A graph illustrating the numbers of cells per well as a function of time was performed to calculate the proliferation rate. Proliferation values

for days 1 to 3 were established as percentages of control (i.e., untreated condition) on day 1. Therefore, the proliferation value of the control on day 1 was established as 100%. Each condition included three replicates (n = 3) for every cell line, and each experiment was repeated independently three times (N = 3).

2.3.2.4. Migration

UCs, RT4 and T24 cells were seeded in 12-well culture plates at 150,000 cells/well density. Cells were incubated for two hours at 37 °C with 8% CO_2 to allow cell adhesion. Then, cells were treated or not with 10^{-8} M BPA or BPS and incubated at 37 °C with 8% CO_2. After 72h, a scratch test was performed [27]. Briefly, a vertical scratch was performed in each well using a 200 µL pipet tip. Wells were rinsed twice to remove detached cells with 3:1 DMEM–Ham F12 medium supplemented with 0.5% fetal bovine serum clone II. Two mL of medium supplemented with 0.5% serum with or without 10^{-8} M BPA or BPS was added to each well. This low serum concentration was chosen to avoid cell proliferation and ensure the observed results are due to cell migration. Cell migration was assessed using a Zeiss Axio Imager M2 Time-Lapse microscope equipped with an AxioCam ICc1 camera (Carl Zeiss, Oberkochen, Germany). Images were processed with the AxioVision 40 V4.8.2.0 software (Carl Zeiss). Photographs were taken every hour for a total of 17 h. Analyses of closure area were measured using ImageJ software (NIH, Bethesda, MD, USA). Migration speed was calculated as the slope of the closure area (Y-axis) as a function of time (X-axis) with the formula “ax + b”, where “a” represents migration speed. The slopes of each cell line were established as percentages of control (i.e., untreated condition). Each condition included two replicates (n = 2) for every cell line, and each experiment was repeated independently three times (N = 3).

2.3.2.5. Flow cytometry

RT4 and T24 cells were seeded in 12-well culture plates at 125,000 cells/well density. Cancer cells were treated with or without 10^{-8} M BPA for ten days and incubated at 37 °C with 8% CO_2. The medium was changed three times a week. After ten days, cells were collected with trypsin, centrifuged at 300 g for 10 min and resuspended in 100 µL of PBS. Cell suspensions

were individually transferred in 100 μL of 3.7% formol to fix cells, and samples were incubated at 4 °C until flow cytometry analyses. Cell samples were washed twice with PBS on the analysis day, followed by blocking using PBS-1% BSA for 45 min at room temperature. Incubation with primary antibody anti-alpha smooth muscle actin (α-SMA) coupled with FITC (1/250 dilution; Abcam) or with isotypic control FITC antibody (1/1000 dilution; Abcam) was performed at room temperature for one hour in PBS-1% BSA. Cells were washed twice with PBS-1% BSA and once with PBS only. Cells were resuspended in 100 μL PBS and samples were analyzed by flow cytometry with a FACSCalibur (Becton Dickson, San Jose, CA, USA) [28]. For each replicate, the value of the isotypic sample was subtracted from the value of the positive sample (cells incubated with antibodies). Each condition included six replicates ($n = 6$), and each experiment was repeated independently three times ($N = 3$).

2.3.2.6. Statistical analysis

Graphical representation and statistical analyses were performed using Microsoft Excel (Microsoft) and GraphPad Prism Software v.9.3 (San Diego, CA, USA). The results are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using the non-parametric tests, the Mann–Whitney test or the Kruskal–Wallis test. Normality analyses were performed for the data in Figure 1 and Figure 7. The values did not meet the required premises to assume normality, which justifies using non-parametric statistical tests. Statistical significance was established at $p < 0.05$.

2.3.3. Results

2.3.3.1. T24 invasive bladder cancer cells exhibit an increased glycolytic capacity compared with RT4 non-invasive bladder cancer cells

Before evaluating the impact of chronic exposure to BPs, the energy metabolism of two UC populations (UC1 and UC2) and two bladder cancer cells (RT4 non-invasive and T24 invasive) was evaluated to compare their glycolytic and mitochondrial capacities. First, UC1 and UC2 cell populations had a similar glycolytic metabolism (**Figure 3.1A-B**). However, UC2 cells exhibited higher OCR levels at basal capacity compared with UC1 cells, whereas the maximal mitochondrial capacity of UC1 and UC2 cells was not significantly different (**Figure 3.1C-D**).

Secondly, RT4 non-invasive bladder cancer cells displayed a significantly higher basal glycolysis than UC1 cells (**Figure 3.1A**). RT4 cells tended to exhibit slightly higher ECAR levels compared with UC2 cells ($p = 0.07$) (**Figure 3.1A**), whereas ECAR levels for maximal glycolytic capacity were comparable with UCs (**Figure 3.1B**). RT4 cells had higher OCR levels for basal mitochondrial respiration than both UCs (**Figure 3.1C**) but exhibited the lowest maximal mitochondrial capacity compared with the other cell lines (**Figure 3.1D**).

Thirdly, T24 invasive bladder cancer cells exhibited the highest ECAR levels for basal and maximal glycolytic capacity compared with the other cell lines (**Figure 3.1A-B**). A general representation of the energy metabolism of T24 cells shows that these cancer cells are highly glycolytic (**Figure 3.S1**). T24 cells exhibit similar OCR levels for basal mitochondrial respiration compared with RT4 cells but significantly higher levels for maximal mitochondrial respiration (**Figure 3.1C-D**). As observed for the glycolytic capacity, T24 cells exhibited significantly higher levels of OCR for maximal mitochondrial respiration compared with RT4 non-invasive bladder cancer cells and UCs. Therefore, initial analyses showed that T24 cells displayed a greater glycolytic capacity than RT4 cells, whereas the two populations of UCs exhibited roughly similar energy metabolism.

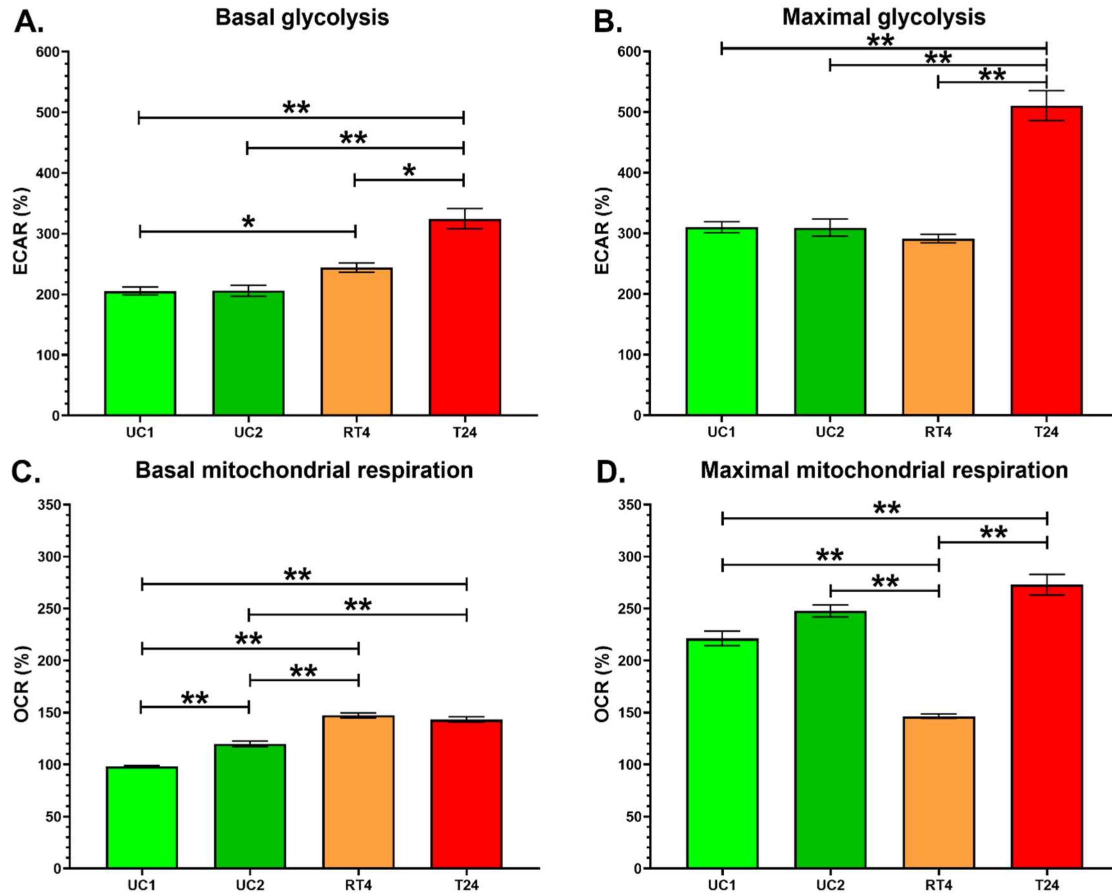

Figure 3.1. T24 invasive bladder cancer cells exhibit an increased glycolytic capacity compared with RT4 non-invasive bladder cancer cells. (**A**, **B**) ECAR and (**C**, **D**) OCR were determined using the XFe96 Extracellular Flux Analyzer in two populations of normal urothelial cells (UC1 and UC2) and non-invasive (RT4) and invasive (T24) bladder cancer cells to establish (**A**) basal glycolysis, (**B**) maximal glycolytic capacity, (**C**) basal mitochondrial respiration and (**D**) maximal mitochondrial capacity. Data are presented as the mean ± SEM and displayed as percentages of UC1 acting as control (n = 10, N = 4). A baseline (100%) was established before injections (see **Figure 3.S1**). * $p < 0.05$, ** $p < 0.01$ by Kruskal–Wallis test.

2.3.3.2. Chronic exposure to physiological concentrations of BPA or BPS does not modulate the glycolysis and mitochondrial respiration of normal urothelial cells

Two UC populations were exposed to physiological concentrations of BPA or BPS to evaluate the impact of these endocrine disruptors on their bioenergetics. In vivo, UCs can be in contact with BPA and BPS through urine [11, 29]. Chronic exposure to 10^{-8} M BPA or BPS did not seem to impact the energy metabolism of UCs. A slight but not significant decrease in ECAR levels was observed for the glycolytic capacity of both compounds (**Figure 3.2A-B**), as well as a subtly decreased OCR level in basal mitochondrial respiration when exposed to BPS ($p = 0.12$) (**Figure 3.2C**). Exposure to BPs did not affect OCR levels associated with maximal mitochondrial respiration (**Figure 3.2D**). Overall, 72 h chronic exposure to physiological concentrations of BPA or BPS did not significantly affect the bioenergetics of normal UCs (**Figure 3.S2**).

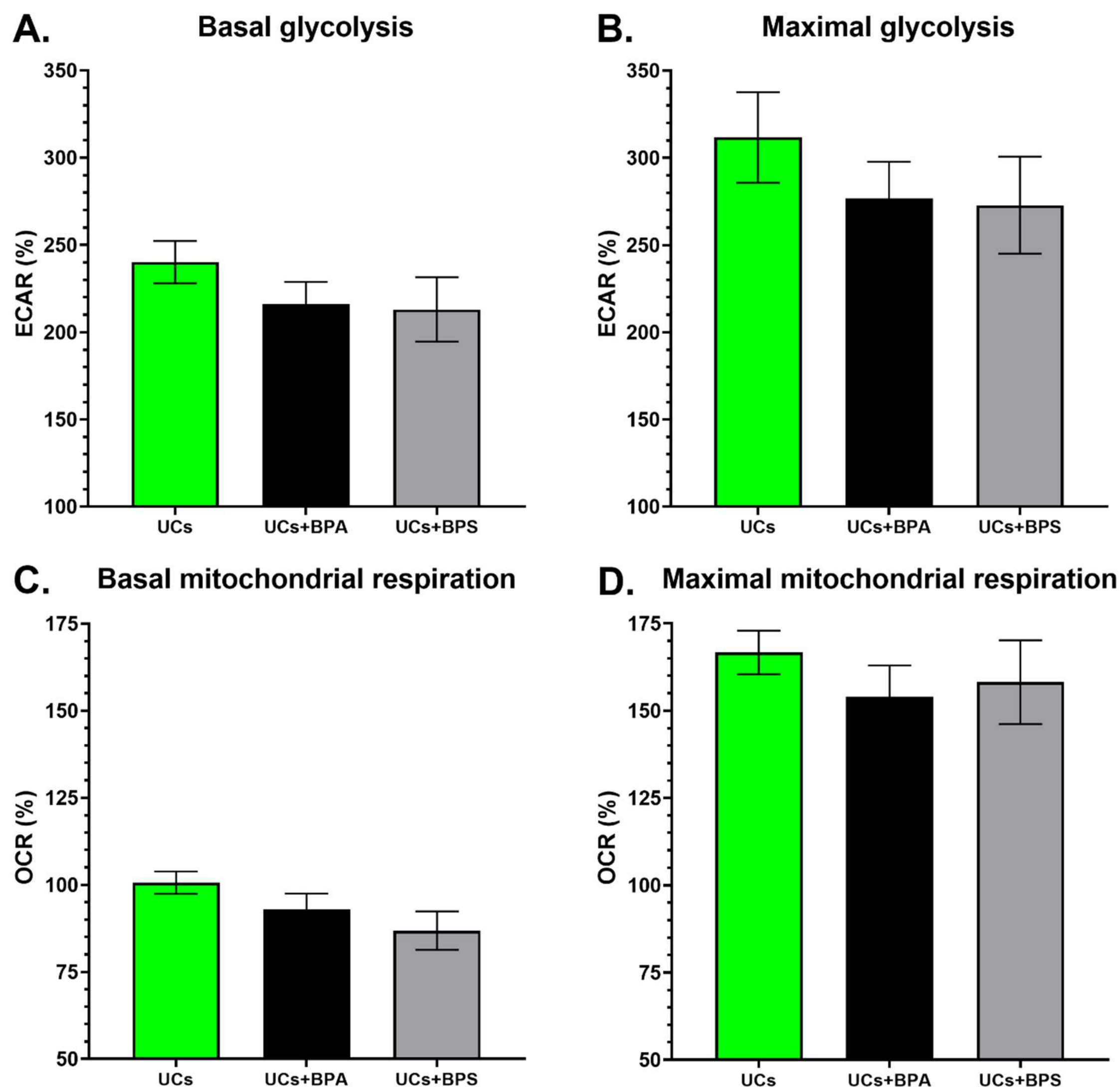

Figure 3.2. Chronic exposure to physiological concentrations of BPA or BPS has no significant effect on the glycolysis and mitochondrial respiration of normal urothelial cells. (**A**, **B**) ECAR and (**C**, **D**) OCR were determined using the XFe96 Extracellular Flux Analyzer for normal urothelial cells (UCs) with or without chronic exposure to physiological concentrations of BPA or BPS to establish (**A**) basal glycolysis, (**B**) maximal glycolytic capacity, (**C**) basal mitochondrial respiration and (**D**) maximal mitochondrial capacity. Analyses represent the results for two populations of normal urothelial cells (UC1 and UC2). Data are presented as the mean ± SEM and displayed as percentages of controls (i.e., untreated condition) ($n \geq 3$, $N = 4$). A baseline (100%) was established before injections (see **Figure 3.S2**). $p < 0.05$ by Mann–Whitney test.

2.3.3.3. RT4 non-invasive bladder cancer cells chronically exposed to physiological concentrations of BPs exhibit increased bioenergetics

RT4 cells were exposed to physiological concentrations of BPA or BPS to evaluate the impact of these endocrine disruptors on non-invasive bladder cancer cells. Like UCs, bladder cancer cells can be exposed to BPs through urine [11, 29]. BPA exposure did not influence the ECAR levels of the basal glycolysis of RT4 cells, whereas chronic exposure of RT4 cells to 10^{-8} M BPS significantly increased the ECAR levels of basal glycolysis (**Figure 3.3A**). Chronic exposure to BPA had a non-significant effect on the ECAR levels associated with the maximal glycolytic capacity. In contrast, exposure to physiological concentrations of BPS tended to increase ECAR levels ($p = 0.07$) (**Figure 3.3B**). RT4 chronically exposed to 10^{-8} M BPA exhibited significantly increased OCR levels associated with basal and maximal mitochondrial respiration (**Figure 3.3C-D**). Although not significantly different from the control condition for mitochondrial metabolism, BPS exposure seemed to have similar biological effects to BPA, suggesting this alternative compound could have comparable effects. Therefore, chronic exposure to physiological concentrations of BPs increased the bioenergetics of RT4 non-invasive bladder cancer cells (**Figure 3.S3**).

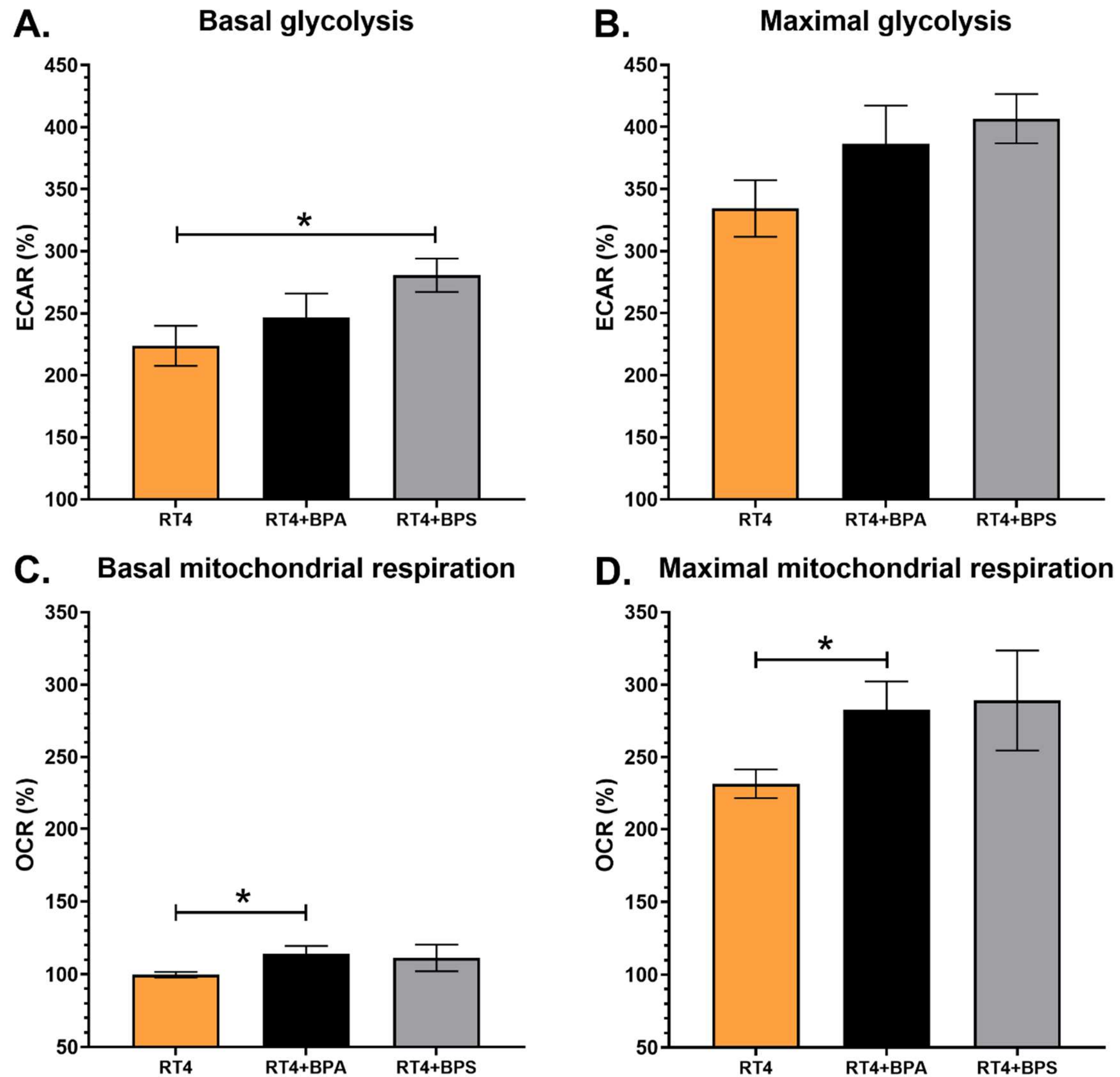

Figure 3.3. RT4 non-invasive bladder cancer cells chronically exposed to physiological concentrations of BPs exhibit increased bioenergetics. (**A**, **B**) ECAR and (**C**, **D**) OCR were determined using the XFe96 Extracellular Flux Analyzer for RT4 non-invasive bladder cancer cells with or without chronic exposure to physiological concentrations of BPA or BPS to establish (**A**) basal glycolysis, (**B**) maximal glycolytic capacity, (**C**) basal mitochondrial respiration and (**D**) maximal mitochondrial capacity. Data are presented as the mean ± SEM and displayed as percentages of controls (i.e., untreated condition) ($n \geq 3$, $N = 3$). A baseline (100%) was established before injections (see **Figure 3.S3**). * $p < 0.05$ by Mann–Whitney test.

2.3.3.4. T24 invasive bladder cancer cells chronically exposed to physiological concentrations of BPA or BPS exhibit an increased glycolytic metabolism

T24 cells were exposed to physiological concentrations of BPA or BPS to evaluate the impact of these compounds on invasive bladder cancer cell metabolism. Chronic exposure to 10^{-8} M BPA or BPS increased the ECAR levels of basal glycolysis of invasive bladder cancer cells (**Figure 3.4A**), whereas only 10^{-8} M BPS significantly increased the ECAR levels of the maximal glycolytic capacity (**Figure 3.4B**). Although not significant, BPA seemed to slightly increase the ECAR levels of the maximal glycolysis of T24 cells ($p = 0.096$) (**Figure 3.4B**). Chronic exposure to BPA and BPS did not affect the OCR levels associated with the basal and maximal mitochondrial respiration of T24 cells (**Figure 3.4C-D**). As previously observed with RT4 cells, BPA and BPS seemed to have similar effects on the energy metabolism of invasive bladder cancer cells (**Figure 3.S4**). Overall, T24 invasive bladder cancer cells exhibited an increased glycolytic metabolism when chronically exposed to physiological concentrations of BPs.

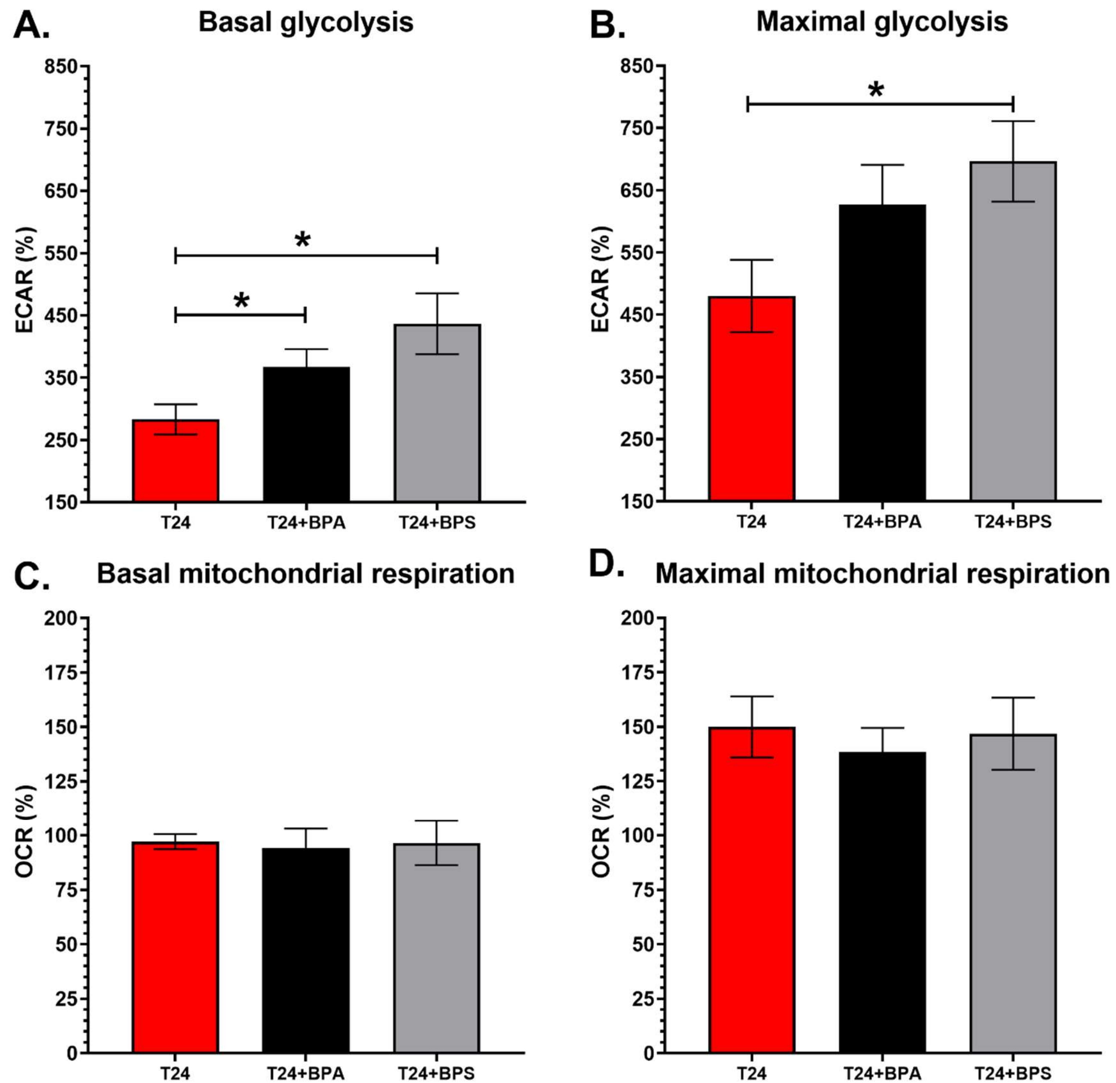

Figure 3.4. T24 invasive bladder cancer cells chronically exposed to BPA or BPS physiological concentrations exhibit an increased glycolytic metabolism. (**A**, **B**) ECAR and (**C**, **D**) OCR were determined using the XFe96 Extracellular Flux Analyzer for T24 invasive bladder cancer cells with or without chronic exposure to physiological concentrations of BPA or BPS to establish (**A**) basal glycolysis, (**B**) maximal glycolytic capacity, (**C**) basal mitochondrial respiration and (**D**) maximal mitochondrial capacity. Data are presented as the mean ± SEM and displayed as percentages of controls (i.e., untreated condition) ($n \geq 3$, $N = 3$). A baseline (100%) was established before injections (see **Figure 3.S4**). * $p < 0.05$ by Mann–Whitney test.

2.3.3.5. Chronic exposure to physiological concentrations of BPA or BPS increases the proliferation rate of RT4 non-invasive bladder cancer cells and induces an initial boost of proliferation for UCs and T24 cells

The proliferation rate of urothelial, RT4 and T24 cells was evaluated for three days under chronic exposure to 10^{-8} M BPA or BPS. First, UCs exposed to BPA or BPS exhibited a significantly higher cell number on days 1 to 3 compared with the control (**Figure 3.5A**). However, it is possible to observe that, following this initial increase, the proliferation rate seemed to stabilize, suggesting that the proliferation rate could be enhanced in the first 24 h of BPA or BPS exposure, resulting in an initial boost. Secondly, RT4 non-invasive bladder cancer cells chronically exposed to 10^{-8} M BPA or BPS exhibited a significantly higher proliferation rate on day 3 than the control (**Figure 3.5B**). However, exposure to BPs did not impact RT4 cell proliferation on days 1 and 2. Thirdly, chronic exposure of T24 cells to BPA or BPS did not affect the proliferation rate on days 2 and 3. However, exposure to BPA significantly increased the proliferation rate of T24 cells on day 1 (**Figure 3.5C**), but its clinical impact is probably not significant. This observation could be associated with an initial increase in proliferation due to BPA exposure since the initial effect observed following BPA or BPS exposure was not maintained on days 2 and 3. Therefore, chronic exposure to physiological concentrations of BPA or BPS increased the proliferation rate of RT4 cells and induced an initial boost of proliferation in the first 24 h for normal UCs and T24 cells.

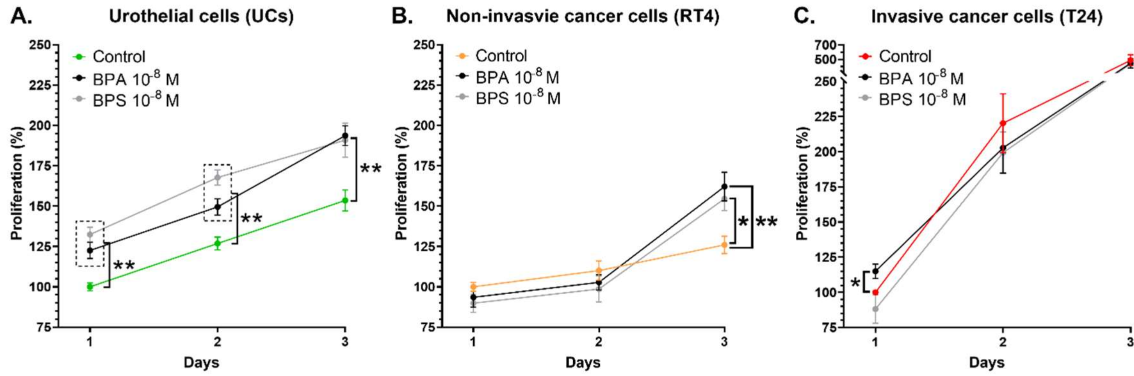

Figure 3.5. Chronic exposure to physiological concentrations of BPA or BPS increases the proliferation rate of RT4 non-invasive bladder cancer cells and induces an initial boost of proliferation for UCs and T24 cells. The proliferation rate of (**A**) normal urothelial cells (UCs), (**B**) RT4 non-invasive bladder cancer cells and (**C**) T24 invasive bladder cancer cells was established over three days with or without chronic exposure to physiological concentrations of BPA or BPS. The proliferation rate is illustrated by the number of cells as a function of time. Data are presented as the mean ± SEM and displayed as percentages of controls at day 1 (i.e., untreated condition) (n = 3, N = 3). * $p < 0.05$, ** $p < 0.01$ by Mann–Whitney test.

2.3.3.6. Chronic exposure to physiological concentrations of BPA or BPS decreases the migration of normal urothelial cells while increasing the migration speed of bladder cancer cells

The migration of urothelial, RT4 and T24 cells was evaluated under chronic exposure to 10^{-8} M BPA or BPS. On the one hand, chronic exposure to physiological concentrations of BPA significantly decreased the migration speed of UCs. In contrast, BPS exposure only resulted in a slight reduction in migration ($p = 0.056$) (**Figure 3.6A**). On the other hand, 10^{-8} M BPA tended to increase the migration speed of RT4 non-invasive bladder cancer cells ($p = 0.06$), but BPS did not affect the migration of RT4 cells (**Figure 3.6B**). BPA and BPS significantly increased the migration speed of T24 invasive bladder cancer cells (**Figure 3.6C**). Therefore, chronic exposure to physiological concentrations of BPA or BPS decreased normal UCs while increasing the migration of T24 invasive bladder cancer cells.

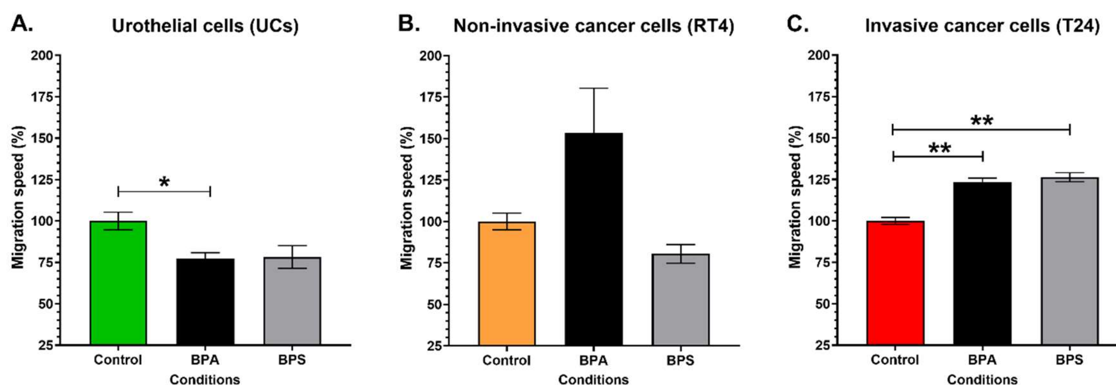

Figure 3.6. Chronic exposure to physiological concentrations of BPA or BPS decreases the migration speed of normal urothelial cells while increasing the migration speed of bladder cancer cells. The migration speed of (**A**) normal urothelial cells (UCs), (**B**) RT4 non-invasive bladder cancer cells and (**C**) T24 invasive bladder cancer cells was evaluated by time-lapse microscopy with or without chronic exposure to physiological concentrations of BPA or BPS. Data are presented as the mean ± SEM and displayed as percentages of controls (i.e., untreated condition) (n = 2, N = 3). The 100% migration value of UCs represents a mean of 12.06 cm^2/h, for RT4 1.68 cm^2/h and for T24 4.77 cm^2/h. * $p < 0.05$, ** $p < 0.01$ by Mann–Whitney test.

2.3.3.7. RT4 non-invasive bladder cancer cells chronically exposed to physiological concentrations of BPA exhibit an increased expression of α-SMA expression

RT4 and T24 cancer cells were chronically exposed to 10^{-8} M BPA and analyzed by flow cytometry to evaluate the impact of this endocrine disruptor on the expression of alpha-smooth muscle actin (α-SMA), which can be used as a cell marker for the invasive potential of cancer cells [30]. Compared with T24 invasive bladder cancer cells, RT4 non-invasive cancer cells expressed significantly lower levels of α-SMA (**Figure 3.7**). However, when chronically exposed to 10^{-8} M BPA, RT4 cells exhibited an increased expression of α-SMA. Furthermore, α-SMA levels of RT4 cells exposed to BPA were not significantly different from the α-SMA levels of T24 cells. The chronic exposure of T24 cells to 10^{-8} M BPA did not substantially affect the α-SMA expression levels. See **Figure 3.S5** for an example of gating analysis. Overall, chronic exposure of RT4 non-invasive bladder cancer cells to physiological concentrations of BPA increased the expression of α-SMA.

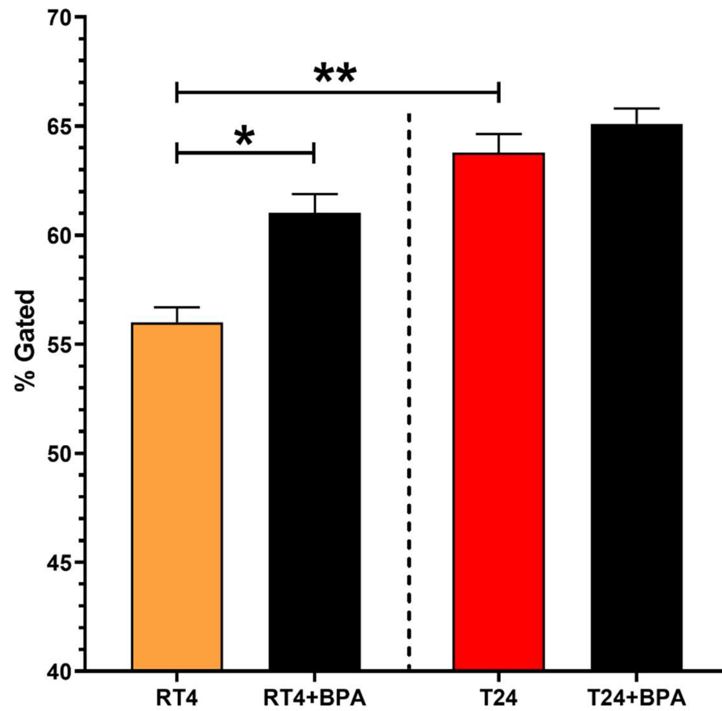

Figure 3.7. RT4 non-invasive bladder cancer cells chronically exposed to BPA's physiological concentrations exhibit an increased α-SMA expression. The expression of α-smooth muscle actin (α-SMA) by RT4 non-invasive and T24 invasive bladder cancer cells was measured by flow cytometry with or without chronic exposure to physiological concentrations of BPA. Data are presented as the mean ± SEM (n = 6, N = 3). * $p < 0.05$, ** $p < 0.01$ by Kruskal–Wallis test.

2.3.4. Discussion

Since the effects of BPs on bladder cancer have not yet been established, the impact of chronic exposure to physiological concentrations of BPA or BPS on normal urothelial cells and non-invasive and invasive bladder cancer cells was studied. Therefore, the effects of BPs on energy metabolism, proliferation, migration and α-SMA expression were examined.

Before evaluating the impact of chronic exposure to physiological concentrations of BPs, the energy metabolism of UCs and RT4 non-invasive and T24 invasive bladder cancer cells was evaluated to compare their glycolytic and mitochondrial capacities. The two different populations of normal UCs (UC1 and UC2) showed similar levels of energy metabolism, and RT4 non-invasive bladder cancer cells displayed higher basal glycolysis and mitochondrial respiration than UCs. In contrast, T24 invasive bladder cancer cells exhibited a greater glycolytic capacity than normal UCs and RT4 non-invasive bladder cancer cells.

Cancer cells typically exhibit an increased metabolic rate due to enhanced physiological activity, characterized by an uncontrolled proliferation rate [31]. Cancer cells are characterized by increased glycolytic metabolism, at the expense of mitochondrial respiration, even in the presence of oxygen and functioning mitochondria [31]. This metabolic switch is called the Warburg effect, a phenomenon that allows cancer cells to have an adapted energy metabolism to support cell growth and proliferation and promote cell invasion [31]. The increased intake of glucose results in a high synthesis of pyruvate. Since the glycolytic rate is superior to the mitochondrial capacity, the excess pyruvate is converted to lactate through the enzyme lactate dehydrogenase [32]. The increased use of the glycolytic pathway results in enhanced lactate production, acidifying the extracellular microenvironment. Lactate can inhibit immune cells, such as monocytes, that are responsible for eliminating unhealthy cells, including cancer [33]. The accumulation of lactate also reorganizes the extracellular matrix, which could promote tumour invasion [22]. However, this enhanced glycolysis was only observed in the RT4 cells for basal glycolysis, not maximal glycolysis. RT4 cells had a significantly higher basal glycolytic metabolism than UC1, but not UC2, and had a similar maximal glycolytic metabolism compared with both UCs. The basal mitochondrial capacity of RT4 cells was increased compared with UCs. The absence

of an enhanced maximal glycolytic capacity when compared with T24 cells could be because RT4 cells are non-invasive, therefore, are less prone to produce an excess of lactate to invade the extracellular matrix.

Furthermore, RT4 cells had a slower doubling rate than T24 cells. The comparison of the proliferation rate of untreated RT4 and T24 cells demonstrates an important difference between both cell lines. RT4 cells have a doubling time of 37 to 66 h [34, 35], whereas T24 cells are reported to have a doubling time of 19 to 24 h [34-36]. Therefore, RT4 non-invasive bladder cancer cells have a lower level of physiological activity, which could explain the absence of metabolic switch observed. Finally, UCs exhibited a similar energy metabolism even though UC2 cells had higher levels of basal mitochondrial respiration. This difference could be due to individual variations associated with genetics or environmental factors, such as drugs, chemical exposure and health [37].

In vivo, the basal layer's UCs are protected from exposure to urine and its contaminants, such as BPs. However, cancer cell growth can alter the impermeability of the urothelium by disrupting cell–cell adhesion [38], thus resulting in the exposure of UCs surrounding the tumour and underlying UCs to urine and potentially BPs. UCs can also be exposed to BPs through the bladder vascular system perfusing the bladder stroma [39]. BPA and BPS had a weak effect on the bioenergetics of UCs. Although not significant, this tendency could be associated with inhibiting certain enzymes related to cell metabolism by BPs. BPA has been shown to inhibit metabolizing enzymes, such as cytochromes P450, glucose transporters and enzymes from the electron transport chain, that can impact some main metabolic pathways, for example, mitochondrial respiration [40, 41]. It is, therefore, essential to confirm the metabolic analyses with physiological parameters to evaluate the functional impact of BPA or BPS on UCs.

The alteration in the energy metabolism of RT4 cells after chronic exposure to BPs could be associated with the multiple binding capacities of BPA and BPS. BPA was shown to bind to ERs with a median inhibitory concentration (IC_{50}) value of 3.3–73 nM [42], which corresponds to the range of concentrations of BPs found in urine (1–100 nM) [11]. Therefore,

the increased energy metabolism observed could be related to the enhanced physiological activity of RT4 cells following exposure to BPs. Although the impact of BPS on mitochondrial capacity was not significantly different from the control condition, chronic exposure to this compound seems to induce similar effects to BPA. These results need to be correlated through the impact of BPA or BPS on the physiological activity of RT4 cells. The relevance of these results depends on the associated biological consequences, for example, proliferation and migration.

Thirdly, T24 invasive bladder cancer cells chronically exposed to physiological concentrations of BPA or BPS did not seem to impact the mitochondrial capacities but exhibited an increased glycolytic metabolism. The increased glycolytic capacity observed in T24 cells following chronic exposure to BPA or BPS could lead to higher lactate production and enhance the acidification of the tumour microenvironment. Although T24 cells are invasive cancer cells, chronic exposure to BPA or BPS could accentuate the consequences of matrix acidification [31], further inhibiting the local immune cells [33], thus facilitating invasion and metastases formation through a major matrix remodelling.

The increased proliferation rate of RT4 non-invasive cancer cells chronically exposed to physiological concentrations of BPA or BPS could suggest a more aggressive phenotype. Indeed, studies have shown that BPA could promote cell proliferation by binding to the GPER in breast cancer cells, which activates the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signalling pathway [43]. Furthermore, BPA can bind to the ERR of endometrial cancer cells, which can activate the epidermal growth factor receptor (EGFR)/extracellular signal-regulated kinase (ERK) pathway associated with proliferation [44]. On the other hand, BPS has also been shown to promote cell proliferation by altering the PI3K/Akt signalling pathway in breast cancer cells [45]. BPA or BPS exposure to T24 invasive bladder cancer cells did not impact the proliferation rate in the long term. Still, BPA induced an increased proliferation in the first 24 h. BPA can alter multiple signalling pathways associated with proliferation, which could explain the increased proliferation rate. However, this difference was not observed on days

2 and 3, which could be explained by the already high proliferation rate of T24 cells, therefore obscuring the impact of BPs in the long term.

Next, the impact of chronic exposure to BPA or BPS on cell migration was evaluated. UCs chronically exposed to physiological concentrations of BPA or BPS exhibited decreased migration. This observation could impact the urothelium tissue repair in the case of disease or bladder injury. Cell migration is essential in wound healing to allow epithelialization and wound closure [46]. The decreased migration capacity of UCs could result in a slower wound closure of the bladder wall, increasing the exposure of underlying tissues to the toxic substances found in urine, such as urea and carcinogens [47]. Chronic exposure to BPA or BPS did not impact RT4 cell migration. RT4 cells have a low migration capacity as these cells form and stay in clusters, resulting in minimal migration. Therefore, the impact of BPs could potentially be obscured by the fact that RT4 cells simply do not tend to migrate. However, chronic exposure to BPA or BPS increased the migration of T24 invasive bladder cancer cells. On the one hand, BPA has been shown to increase the migration of lung cancer cells through the GPER/EGFR/ERK1/2 signalling pathway [48] and increase the migration of triple-negative breast cancer cells through ERR [7]. Studies by Derouiche et al. demonstrated that exposure to BPA can promote the cell migration of prostate cancer cells through the modulation of ion channel protein expression associated with calcium entry [8]. On the other hand, exposure to BPS has been shown to promote the migration of human non-small cell lung cancer cells through ERK1/2, mediated by the transforming growth factor β (TGF-β)/Smad-2/3 signalling pathway [11]. Deng et al. have reported that BPS can also promote the migration of triple-negative breast cancer cells in vitro through the GPER/Hippo signalling pathway, resulting in the activation and nuclear accumulation of yes-associated protein (YAP), thus upregulating downstream genes [49]. Therefore, these results concur with the literature and the metabolic analyses, demonstrating an enhanced energy metabolism in T24 cells when exposed to BPA or BPS.

In brief, chronic exposure to physiological concentrations of BPs tended to decrease the energy metabolism of UCs, increase their proliferation in the first 24 h and decrease their migration. In addition, exposure to BPs increased the energy metabolism and the proliferation

of non-invasive RT4 bladder cancer cells but had little to no effect on their migration. Finally, exposure to BPs increased the glycolytic capacity and the migration of invasive T24 bladder cancer cells but did not impact their mitochondrial respiration or proliferation (Table 1). These results suggest that chronic exposure to BPA and BPS alters the energy metabolism and behaviours of UCs and non-invasive and invasive bladder cancer cells, which could potentially promote bladder cancer progression.

Cell types	**Parameters**		**BPA**	**BPS**
UCs	Glycolysis	Basal	↓	↓
		Maximal	↓	↓
	Mitochondrial respiration	Basal	Ø	↓
		Maximal	Ø	Ø
	Proliferation		↑	↑
	Migration		↓↓	↓
RT4 cells	Glycolysis	Basal	Ø	↑↑
		Maximal	Ø	↑
	Mitochondrial respiration	Basal	↑↑	↑
		Maximal	↑↑	↑
	Proliferation		↑↑	↑↑
	Migration		↑	Ø
T24 cells	Glycolysis	Basal	↑↑	↑↑
		Maximal	↑	↑↑
	Mitochondrial respiration	Basal	Ø	Ø
		Maximal	Ø	Ø
	Proliferation		Ø	Ø
	Migration		↑↑	↑↑

Legend: Ø = No impact; ↑/↓ = Tendency; ↑↑/↓↓ = Significant

Table 3.1. Impact of chronic exposure to physiological concentrations of BPA and BPS on the energy metabolism, proliferation and migration of UCs and RT4 non-invasive and T24 invasive bladder cancer cells. UCs are generally negatively affected by exposure to BPs, illustrated by the decreased bioenergetics and migration. Conversely, exposure to BPs generally enhances the bioenergetics, proliferation and migration of cancer cells.

The impact of chronic exposure to BPA on the α-SMA expression of RT4 and T24 cells was established. α-SMA can be used as an epithelial–mesenchymal transition (EMT) marker, during which α-SMA expression is increased [30]. When cancer cells transit from a non-invasive to an invasive and metastatic phenotype, they undergo various steps, including EMT [50]. Increased α-SMA expression is associated with an invasive phenotype and a greater capacity to produce metastases [30], therefore resulting in a poorer prognosis in multiple cancers such as lung [51] and breast cancer [52]. Consequently, invasive cancer cells tend to express higher levels of α-SMA than non-invasive cancer cells [30], allowing α-SMA expression to be used as a cell marker for cancer cell aggressiveness. The results show that RT4 non-invasive bladder cancer cells expressed significantly lower levels of α-SMA when compared with T24 invasive bladder cancer cells. Chronic exposure of RT4 cells to a physiological concentration of BPA increased the expression of α-SMA. Chronic exposure to BPA could, therefore, enhance the aggressiveness of non-invasive cancer cells, thus promoting the transition from a non-invasive to an invasive phenotype. Clinically, these results suggest that chronic exposure to BPs could promote bladder cancer recurrence and progression.

This study is not without limitations. First, the inability to ensure a bisphenol-free control represents a limitation [53]. Since bisphenol-based plastics are abundantly used in laboratory equipment and scientific instruments, it is challenging to avoid external bisphenol contamination. However, there were still significant differences between controls and the BPA or BPS conditions. Second, using an in vitro 2D cell culture is a notable limitation. Using a 3D bladder cancer model [54] would better represent the physiological impact of chronic exposure to BPA or BPS on UCs and bladder cancer tumours. RT4 and T24 cells were selected in this study for their ability to form spheroids, as we are planning to further investigate the effects of BPs using our 3D bladder cancer model [54].

Measuring the presence of BPs in different stages of bladder cancer samples would be an interesting perspective for this study to determine if advanced bladder cancers exhibit higher levels of BPs than low-grade bladder cancer patients. Furthermore, evaluating the impact of BPs' metabolites on bladder cancer progression would be valuable. A significant proportion

of BPs is metabolized and excreted through urine [55]. However, studies have shown that these metabolites can remain active in the body [56], suggesting they could also potentially affect cancer development. Unfortunately, the impact of BPs' metabolites has never been investigated in bladder cancer.

2.3.5. Conclusions

Few studies have evaluated the potential impact of chronic exposure to BPs on bladder cancer, despite the presence of these endocrine disruptors in human urine and the accumulating literature demonstrating their pro-tumorigenic capacities. This study has brought valuable insights into the effects of chronic exposure to a physiological concentration of BPA or BPS on bladder cancer progression. The impact of these BPs on the energy metabolism, proliferation, migration and α-SMA expression of normal UCs and RT4 non-invasive and T24 invasive bladder cancer cells was established. The results show that chronic exposure to BPA or BPS increases the proliferation rate of UCs while decreasing their migration, which could result in hyperplasia and altered wound healing capacities. Exposure to BPA or BPS also increases cancer cells' energy metabolism and physiological activity, particularly the metabolism, proliferation and α-SMA expression of RT4 non-invasive bladder cancer cells, which could promote bladder cancer progression from a non-invasive to an invasive phenotype. The ubiquitous and continuous exposure to these endocrine disruptors, through food and the environment, could have a meaningful clinical impact on bladder cancer recurrence, progression and patient prognosis.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/cancers14164011/s1, Figure S1: Glycolytic and mitochondrial metabolism of normal urothelial cells (UC1 and UC2) and non-invasive (RT4) and invasive (T24) bladder cancer cells; Figure S2: Impact of BPA and BPS on the glycolytic and mitochondrial metabolism of two populations of normal urothelial cells (UC1 and UC2); Figure S3: Impact of BPA and BPS on the glycolytic and mitochondrial metabolism of RT4 non-invasive bladder cancer cells; Figure S4: Impact of BPA and BPS on the glycolytic and mitochondrial metabolism of T24 invasive bladder cancer cells; Figure S5: Example of gating analysis for the impact of BPA on the expression of α-SMA of RT4 cells.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the CHU de Québec (protocol code 2012-1341, DR-002-1190, approved and renewed annually since December 2013).

Informed Consent Statement: Informed consent was obtained from all subjects who provided tissue samples for this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

2.3.6 Supplementary material

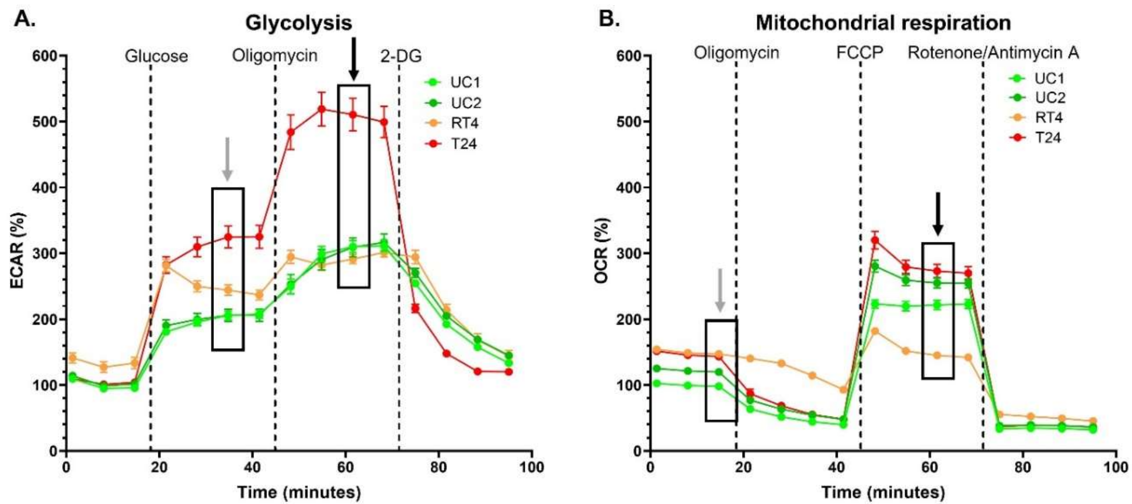

Figure 3.S1. Glycolytic and mitochondrial metabolism of normal urothelial cells (UC1 and UC2) and non-invasive (RT4) and invasive (T24) bladder cancer cells. (**A**) The glycolytic metabolism was established by the sequential injections of glucose, oligomycin and 2-DG. Analyses in Figure 1A-B were performed using measure #6 (gray arrow) for basal glycolysis and measure #10 (black arrow) for maximal glycolytic capacity. (**B**) The mitochondrial respiration was established by sequential injections of oligomycin, FCCP and the combination of rotenone and antimycin A. Analyses in Figure 1C-D were performed using measure #3 (gray arrow) for basal mitochondrial respiration and measure #10 (black arrow) for maximal mitochondrial respiration. Data are displayed as percentages of UC1 acting as control (n = 10, N = 4). The baseline (100%) was established before the first injection, namely before glucose injection for the glycolytic capacity and before oligomycin injection for the mitochondrial respiration.

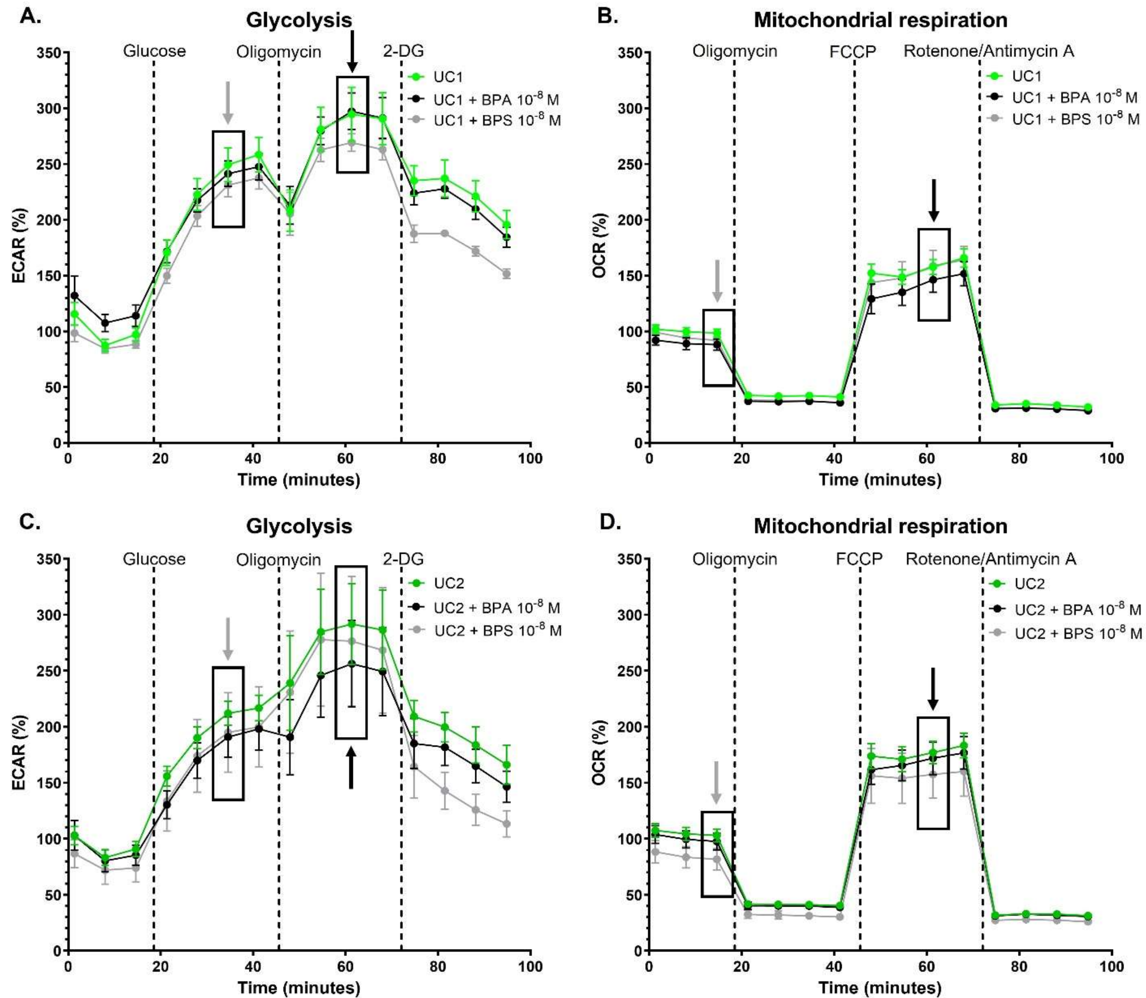

Figure 3.S2. Impact of BPA and BPS on the glycolytic and mitochondrial metabolism of two populations of normal urothelial cells (UC1 and UC2). (**A, C**) The glycolytic metabolism was established by the sequential injections of glucose, oligomycin and 2-DG. Analyses in Figure 2 represent the results' combination of UC1 and UC2. Analyses in Figure 2A-B were performed using measure #6 (gray arrow) for basal glycolysis and measure #10 (black arrow) for maximal glycolytic capacity. (**B, D**) The mitochondrial respiration was established by sequential injections of oligomycin, FCCP and the combination of rotenone and antimycin A. Analyses in Figure 2C-D were performed using measure #3 (gray arrow) for basal mitochondrial respiration and measure #10 (black arrow) for maximal mitochondrial respiration. Data are displayed as percentages of controls (i.e., untreated condition) (n = 3, N = 4). The baseline (100%) was established before the first injection, namely before glucose injection for the glycolytic capacity and before oligomycin injection for the mitochondrial respiration.

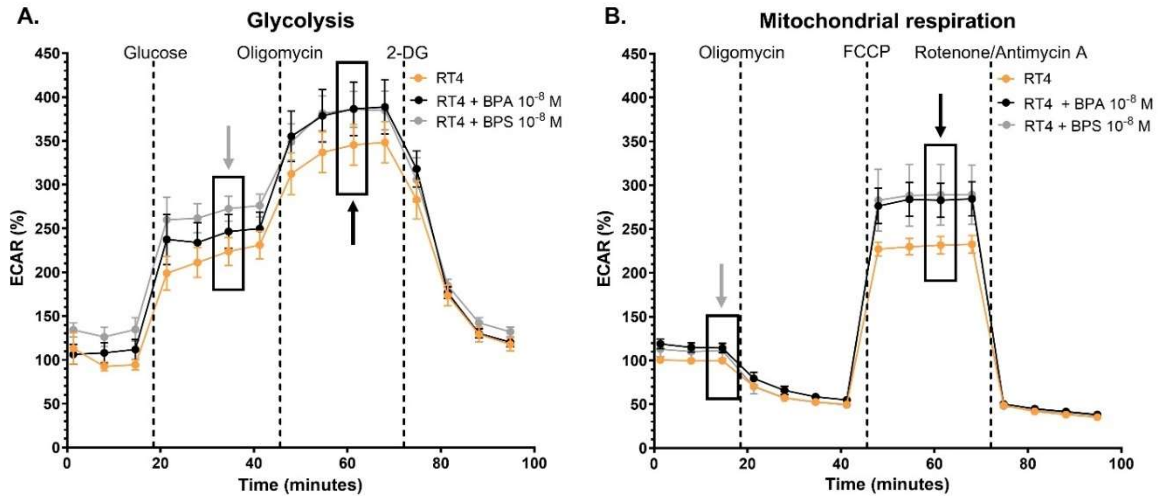

Figure 3.S3. Impact of BPA and BPS on the glycolytic and mitochondrial metabolism of RT4 non-invasive bladder cancer cells. (**A**) The glycolytic metabolism was established by the sequential injections of glucose, oligomycin and 2-DG. Analyses in Figure 3A-B were performed using measure #6 (gray arrow) for basal glycolysis and measure #10 (black arrow) for maximal glycolytic capacity. (**B**) The mitochondrial respiration was established by sequential injections of oligomycin, FCCP and the combination of rotenone and antimycin A. Analyses in Figure 3C-D were performed using measure #3 (gray arrow) for basal mitochondrial respiration and measure #10 (black arrow) for maximal mitochondrial respiration. Data are displayed as percentages of controls (i.e., untreated condition) (n = 3, N = 3). The baseline (100%) was established before the first injection, namely before glucose injection for the glycolytic capacity and before oligomycin injection for the mitochondrial respiration.

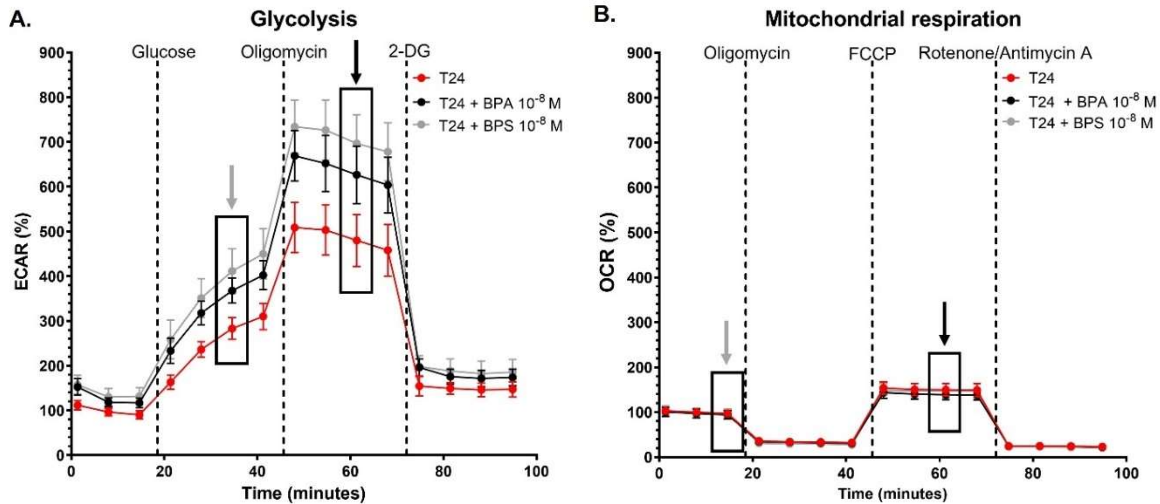

Figure 3.S4. Impact of BPA and BPS on the glycolytic and mitochondrial metabolism of T24 invasive bladder cancer cells. (**A**) The glycolytic metabolism was established by the sequential injections of glucose, oligomycin and 2-DG. Analyses in Figure 4A-B were performed using measure #6 (gray arrow) for basal glycolysis and measure #10 (black arrow) for maximal glycolytic capacity. (**B**) The mitochondrial respiration was established by sequential injections of oligomycin, FCCP and the combination of rotenone and antimycin A. Analyses in Figure 4C-D were performed using measure #3 (gray arrow) for basal mitochondrial respiration and measure #10 (black arrow) for maximal mitochondrial respiration. Data are displayed as percentages of controls (i.e., untreated condition) (n = 3, N = 3). The baseline (100%) was established before the first injection, namely before glucose injection for the glycolytic capacity and before oligomycin injection for the mitochondrial respiration.

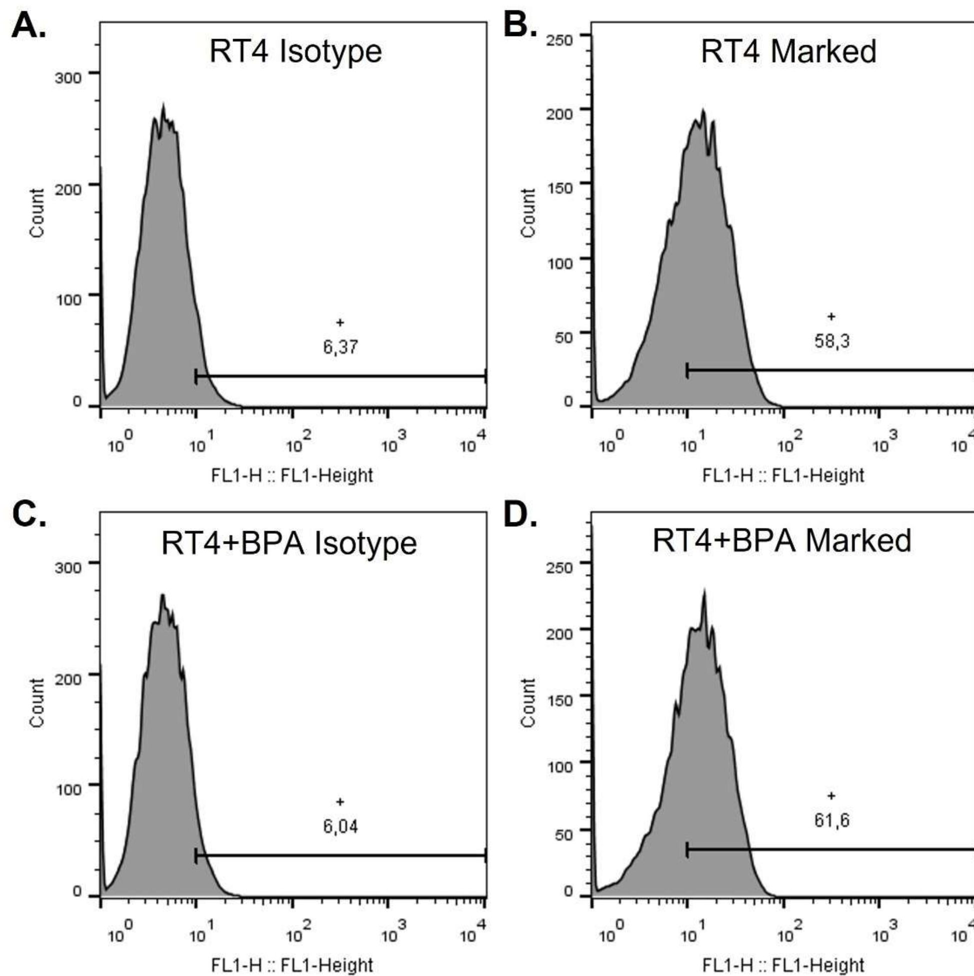

Figure 3.S5. Example of gating analysis for the impact of BPA on the expression of α-SMA of RT4 cells. The gating of α-SMA positive cells was established by subtracting the positive cells marked with the isotype control (**A**, **C**) to the positive cells marked with the anti-α-SMA antibody (**B**, **D**). This example illustrates the gating established for one replicate of RT4 cells (**A**, **B**) and one replicate of RT4 chronically exposed to physiological concentrations of BPA (**C**, **D**).

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Chapitre 3 : Le bisphénol A altère le métabolisme énergétique des cellules stromales et pourrait promouvoir la progression du cancer de la vessie

3.1 Résumé

Le bisphénol A (BPA) est un perturbateur endocrinien présent dans les plastiques. Via sa libération dans les aliments et l'environnement, le BPA est absorbé par l'organisme et est principalement excrété dans l'urine. La vessie y est donc continuellement exposée. Le BPA peut altérer plusieurs voies de signalisation associées à la prolifération, la migration et l'invasion. Nos résultats démontrent que l'exposition au BPA diminue le métabolisme énergétique des fibroblastes vésicaux, ce qui pourrait altérer la matrice extracellulaire. De plus, l'induction des fibroblastes en fibroblastes associés au cancer (CAF) induit une reprogrammation métabolique similaire à l'effet de Warburg. De plus, l'exposition au BPA exacerbe cette reprogrammation métabolique observée chez les CAF, via une amplification du métabolisme glycolytique, ce qui entraine une acidification de l'environnement extracellulaire. Ces résultats suggèrent qu'une exposition chronique au BPA pourrait favoriser la progression du cancer de la vessie via une altération du métabolisme énergétique des cellules stromales.

3.2 Abstract

Bisphenol A (BPA) is an endocrine-disrupting molecule used in plastics. Through its release in food and the environment, BPA can be found in humans and is mostly excreted in the urine. The bladder is therefore continuously exposed to this compound. BPA can bind to multiple cell receptors involved in proliferation, migration, and invasion pathways, and exposure to BPA is associated with cancer progression. Considering the physiological concentrations of BPA in urine, we tested the effect of nanomolar concentrations of BPA on the metabolism of bladder fibroblasts and cancer-associated fibroblasts (CAFs). Our results show that BPA led to a decreased metabolism in fibroblasts, which could alter the extracellular matrix. Furthermore, CAF induction triggered a metabolic switch, similar to the Warburg effect described in cancer cells. Additionally, we demonstrated that nanomolar concentrations of BPA could exacerbate this metabolic switch observed in CAFs via an increased glycolytic metabolism, leading to greater acidification of the extracellular environment. These findings suggest that chronic exposure to BPA could promote cancer progression through an alteration of the metabolism of stromal cells.

3.3 Article

Bisphenol A Alters the Energy Metabolism of Stromal Cells and Could Promote Bladder Cancer Progression

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Conflicts of Interest: The authors declare no conflict of interest.

Simple Summary: Our research brings new insight on the potential impact of bisphenol A on bladder cancer progression. By evaluating the effects of bisphenol A on the stromal environment of bladder cancer, we aimed to demonstrate that this endocrine disruptor could promote bladder cancer invasion through alteration of the energy metabolism of stromal cells, specifically on bladder fibroblasts and cancer-associated fibroblasts. These findings could modify the understanding of bladder cancer since bladder tissue is not recognized as a hormone-sensitive tissue. Consequently, our study suggests that endocrine disruptors, such as bisphenol A, could impact bladder cancer progression.

3.3.1. Introduction

Bisphenol A (BPA) is a chemical compound used to produce plastics, such as polycarbonate, polyesters and epoxy resins [1]. It is used in a wide variety of daily products, such as water bottles and food containers. BPA molecules can be released in food and the environment [1], which causes humans and animals to be continuously exposed. Once in the organism, BPA is partially metabolized and excreted, mainly via urine [2]. It is possible to detect measurable concentrations of BPA in more than 90% of urine samples from humans [3]. Due to its molecular structure being similar to that of estrogen, BPA binds to multiple cellular receptors such as estrogen receptors (ERs), the androgen receptor (AR) and the G-protein-coupled estrogen receptor (GPER) [1]. Thus, acting as an endocrine disruptor, BPA affects signaling pathways related to proliferation [4], cell migration [4,5], invasion [5] and apoptosis [6]. BPA exposure is also associated with cancer development, especially for hormone-dependent cancers such as breast [5] and prostate cancer [7].

Bladder tissue is not recognized as a hormone-dependent tissue, but studies have shown that ERs and the AR are involved in bladder cancer initiation and progression [8,9]. Considering the presence of BPA in urine and the presence of steroid receptors in the bladder urothelium, BPA could have a potential role in bladder cancer development [10].

A hallmark of tumors is the alteration of the metabolic profile of cancer cells, known as the Warburg effect, which consists of a metabolic switch from mitochondrial respiration to glycolytic metabolism [11]. Aerobic glycolysis allows cancer cells to synthesize ATP faster, although providing less overall ATP generation, since the production of lactate from glucose is much faster than the complete oxidation of glucose through the mitochondria [12]. Furthermore, the Warburg effect leads to the production of excess biosynthetic intermediates in the cell, allowing it to generate proteins, lipids and nucleotides, therefore supporting the increased proliferation of cancer cells [13]. Additionally, the transformation of glucose to lactate allows the cell to regenerate NAD+, which is essential to maintain glycolysis [11]. From the point of view of the tumor microenvironment (TME), the Warburg effect can be favorable for cancer cells. The increased glycolytic metabolism leads to higher lactate production, therefore decreasing the local pH and provoking acidification of the TME [11].

This acidification can be beneficial for cancer cells; for example, it alters the stroma, which promotes cancer cell invasion and metastasis [14].

The TME is characterized by multiple cell types, including ones from the epithelium and the stroma, that communicate and interact with each other [15]. Among them, cancer-associated fibroblasts (CAFs) play a critical role in cancer progression. Studies by Ringuette-Goulet et al. have shown that invasive bladder cancer cells release TGF-β, which induces normal fibroblasts into CAFs [16]. In turn, CAFs secrete IL-6, which increases the epithelial–mesenchymal transition of non-invasive bladder cancer cells and could allow cell invasion in the stroma [17].

Considering the omnipresence of BPA in urine and its impact as an endocrine disruptor through ERs and the AR, we hypothesized that BPA would impact the metabolism of healthy and cancer-associated bladder fibroblasts, which could promote bladder cancer progression.

3.3.2. Materials and Methods

3.3.2.1. Cell Lines

All procedures involving patients were conducted according to the Helsinki Declaration and were approved by the local Research Ethical Committee. Donors' consent for tissue harvesting was obtained for each specimen, and experimental procedures were performed according to the CHU de Québec guidelines. Human bladder fibroblasts (HBFs) were extracted from a normal human urological tissue biopsy and were cultured as previously described [18]. The HBFs used were all from the same patient (not transformed primary cell line).

HBFs, non-invasive RT4 bladder cancer cells (ATCC HTB-2) and invasive T24 bladder cancer cells (ATCC HTB-4) were used. Cells were grown in culture media composed of a 3:1 mix of the Dulbecco-Vogt modification of Eagle's medium (DMEM) (Invitrogen, Burlington, ON, Canada) and Ham F12 medium (Invitrogen) supplemented with 5% fetal bovine serum clone II (HyClone, GE Healthcare Life Science, Wauwatosa, WI, USA), 24.3

μg/mL adenine (Corning, Tewksbury, MA, USA), 10 ng/mL epidermal growth factor (Austral Biologicals, San Ramon, CA, USA), 0.212 μg/mL isoproterenol (Sandoz, Boucherville, QC, Canada), 5 μg/mL insulin (Sigma-Aldrich, Oakville, ON, Canada), 0.4 mg/mL hydrocortisone (Calbiochem, San Diego, CA, USA), 100 U/mL penicillin (Sigma-Aldrich) and 25 mg/mL gentamicin (Schering-Plough Canada Inc./Merck, Scarborough, ON, Canada) and incubated at 37 °C with 8% CO_2. Media were changed three times per week.

3.3.2.2. CAF Induction

HBFs were seeded in 96-well Seahorse XF cell culture plates (Agilent/Seahorse Bioscience, Chicopee, MA, USA) 72 h before CAF induction to allow cell adhesion and confluence, thus limiting cell proliferation during treatment. RT4 and T24 bladder cancer cells were seeded in 6-well plates and exposed or not to 10^{-8} M BPA (Millipore Sigma, Oakville, ON, Canada) for 72 h. At day 0 of CAF induction, conditioned medium from RT4 or T24 ± BPA was collected and centrifuged at 300× *g* for 10 min and added to HBF cultures. BPA was also directly added in some HBF culture conditions to study the direct exposure. Exposure to conditioned media was maintained for 8 days to ensure CAF induction [16]. HBFs were also directly exposed to 10^{-8} M BPA in parallel to CAF induction, and HBFs without BPA or conditioned media were used as controls (**Figure 4.1**). Media were changed three times per week. Bioenergetic parameters were measured on day 8. CAF induction was confirmed by α-smooth muscle actin (α-SMA) expression through FACS analysis, as previously demonstrated [16].

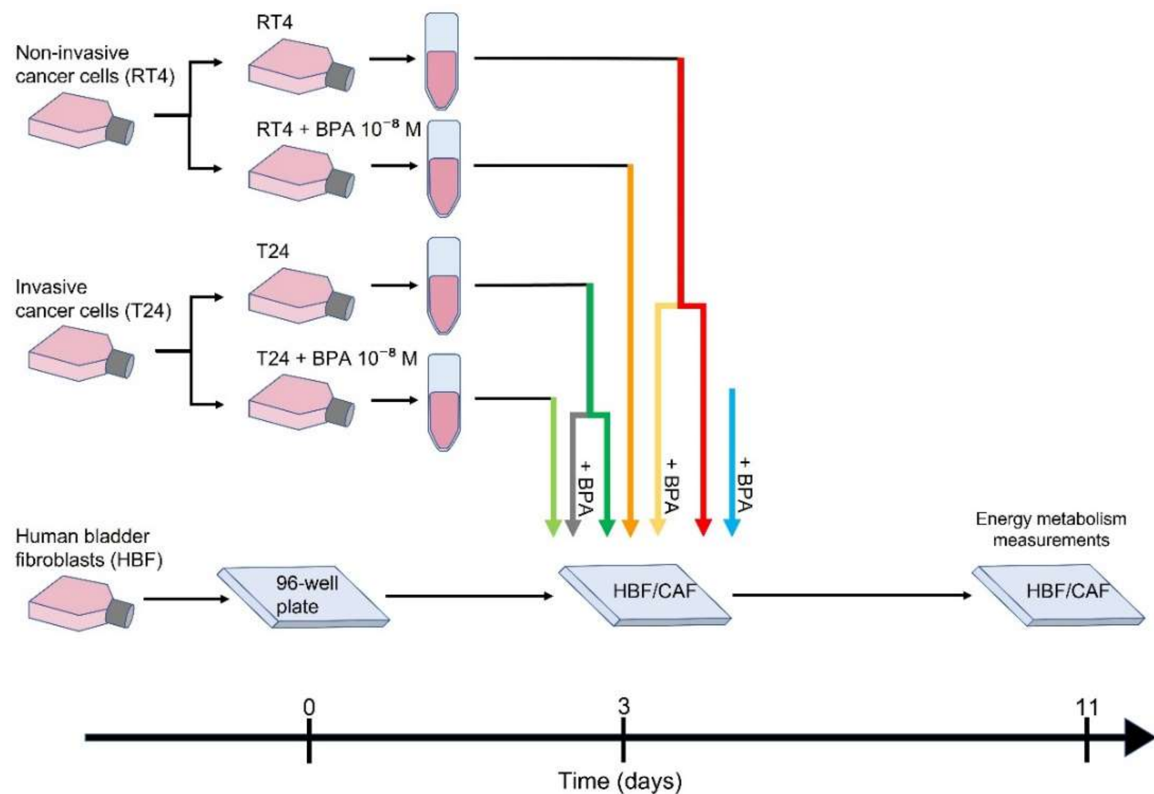

Figure 4.1. Experimental design. Non-invasive bladder cancer cells (RT4) and invasive bladder cancer cells (T24) were cultured and exposed or not to 10^{-8} M BPA for 72 h to mimic its presence in urine (indirect exposure). Human bladder fibroblasts (HBFs) were seeded in a 96-well Seahorse XF cell culture plate. After 72 h, cancer cell-conditioned media were collected, centrifuged and added to HBFs for induction into cancer-associated fibroblasts (CAFs). Healthy HBFs were used as controls. Furthermore, 10^{-8} M BPA was also added to HBFs and HBF/CAFs conditioned with RT4/T24 media to mimic direct exposure of the stroma to BPA. After 8 days of exposure to conditioned media in the presence or absence of BPA, energy metabolism was measured using the XFe Extracellular Flux Analyzer via glycolysis and mitochondrial respiration.

3.3.2.3. Seahorse Energy Metabolism Measurements

Seahorse XFe96 sensor cartridge plates (Agilent/Seahorse Bioscience) were hydrated the day before the analysis with the XF Calibrant (Agilent/Seahorse Bioscience) and incubated at 37 °C without CO_2 overnight. Before the energy metabolism measurements, cells were washed and incubated for 1 h with Glyco Stress media or Mito Stress media. Glyco Stress media

consisted of XF Base Medium (minimal DMEM) (Agilent/Seahorse Bioscience) supplemented with 2 mM L-glutamine (Wisent Bioproducts Inc., Saint-Jean-Baptiste, QC, Canada). Mito Stress media consisted of XF Base Medium supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate (Wisent Bioproducts Inc.) and 10 mM D-(+)-glucose (Millipore Sigma). The extracellular acidification rate (ECAR), representative of glycolytic metabolism, and the oxygen consumption rate (OCR), representative of mitochondrial respiration, were determined using the XFe Extracellular Flux Analyzer (Agilent/Seahorse Bioscience) [19]. The glycolytic metabolism was established by the sequential injection of 10 mM D-(+)-glucose (Millipore Sigma), 1.5 µM of the ATP synthase inhibitor oligomycin (Cayman Chemical, Ann Arbor, MI, USA) to inhibit mitochondrial respiration and force the cells to maximize their glycolytic capacity and 50 mM 2-deoxy-D-glucose (2-DG) (Alfa Aesar, Ward Hill, MA, USA), a competitive inhibitor of the first step of glycolysis. The mitochondrial respiration was measured by the sequential injection of 1.5 µM of the ATP synthase inhibitor oligomycin (Cayman Chemical), 0.5 µM of the mitochondrial uncoupler trifluoromethoxy carbonylcyanide phenylhydrazone (FCCP) (Cayman Chemical) and a combination of 0.5 µM of the mitochondrial complex I inhibitor rotenone (MP Biomedicals, Santa Ana, CA, USA) and 0.5 µM of the mitochondrial complex III inhibitor antimycin A (Millipore Sigma). The concentrations indicated for each injection represent the final concentrations in the wells. At least three measurement cycles (3 min of mixing + 3 min of measuring) were completed before and after each injection. The OCR and ECAR were calculated using Wave software v2.6 (Agilent/Seahorse Bioscience). Energy metabolism was normalized according to the number of cells using a CyQuant Cell proliferation assay kit (Invitrogen) following the manufacturer's instructions. The fluorescence of each well was measured at 485 nm/535 nm for 0.1 s using the Victor2 1420 MultiLabel Counter plate reader (Perkin Elmer Life Sciences, Waltham, MA, USA) and Wallac 1420 software (PerkinElmer). The normalization values were calculated from the fluorescence measurements with Microsoft Excel software (Microsoft, Redmond, WA, USA) and applied to the metabolic values. Each experiment included eight replicates ($n = 8$), and each experiment was repeated three times ($N = 3$).

3.3.2.4. Statistical Analysis

Graphical representation and statistical analyses were performed using GraphPad Prism Software v.9.2 (San Diego, CA, USA). The results are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using the unpaired Student's *t*-test or one-way analysis of variance (ANOVA). Statistical significance was established at $p < 0.05$.

3.3.3. Results

3.3.3.1. Healthy Human Bladder Fibroblasts Exhibit Decreased Glycolytic and Mitochondrial Metabolism following Chronic Exposure to BPA

Human bladder fibroblasts (HBFs) were exposed to physiological concentrations of BPA to evaluate the impact of this compound on these cells. In vivo, BPA can reach HBFs through blood vessels nourishing the cells populating the stroma and potentially affects them. HBFs chronically exposed to 10^{-8} M BPA exhibited a generally decreased energy metabolism compared to untreated HBFs (**Figure 4.2A, D**). HBFs exposed to BPA demonstrated a significantly reduced basal glycolytic metabolism (**Figure 4.2B**), while maximal glycolysis slightly decreased, with a *p*-value established at 0.0528 (**Figure 4.2C**). HBFs exposed to BPA also exhibited significantly decreased basal and maximal mitochondrial respiration (**Figure 4.2E, F**). Therefore, HBFs chronically exposed to physiological concentrations of BPA are characterized by a reduced glycolytic and mitochondrial oxidative metabolism.

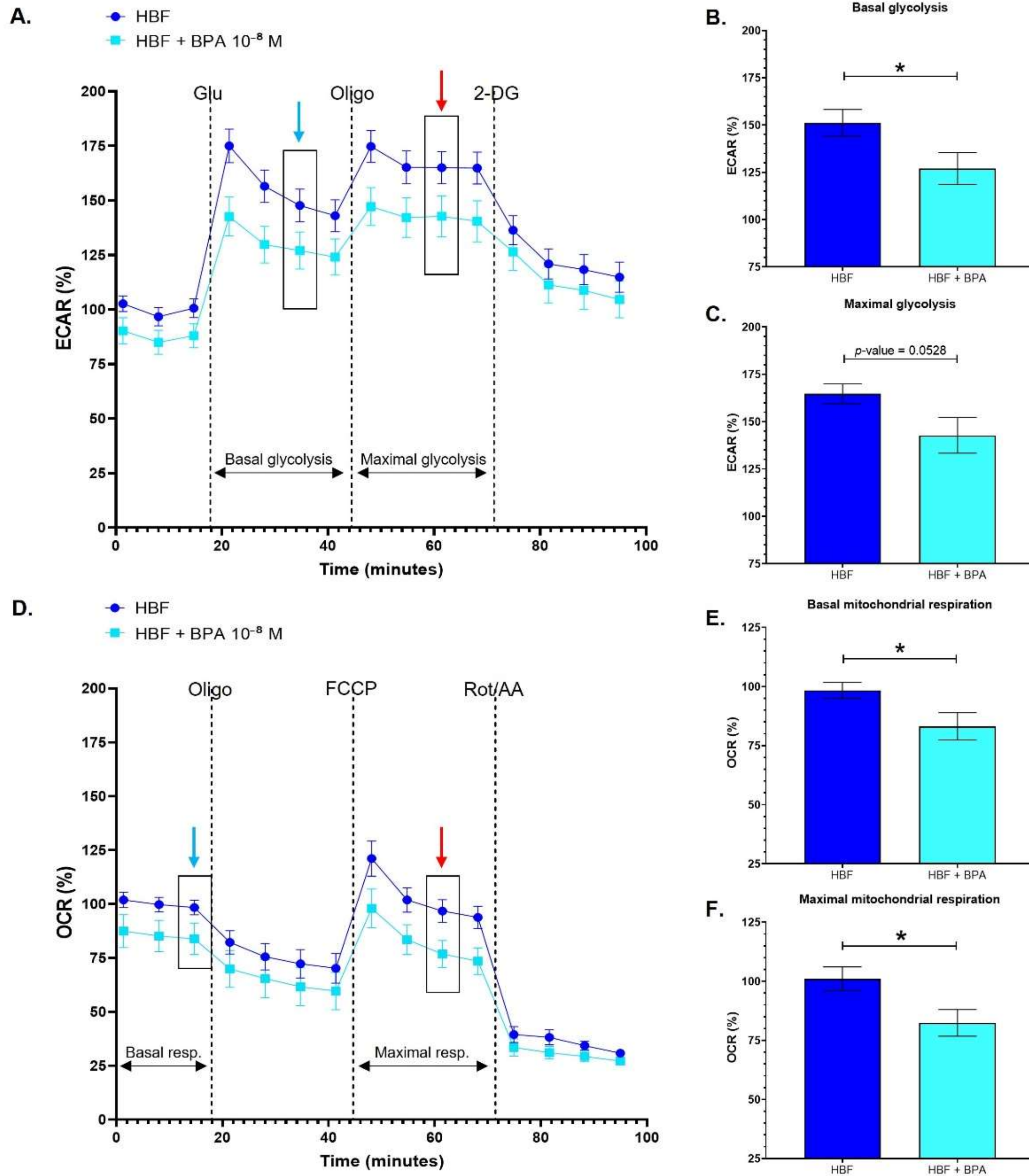

Figure 4.2. Healthy human bladder fibroblasts exhibit a decreased glycolytic and mitochondrial metabolism following chronic exposure to BPA. (**A**–**C**) ECAR and (**D**–**F**) OCR were determined using the XFe96 Extracellular Flux Analyzer in healthy human bladder fibroblasts exposed (HBF + BPA) or not (HBF) to 10^{-8} M BPA for 8 days to establish (**B**) basal glycolysis, (**C**) maximal glycolytic capacity, (**E**) basal mitochondrial respiration and (**F**) maximal mitochondrial capacity. (**A**) The glycolytic metabolism was established by sequential injections of glucose (Glu), oligomycin (Oligo) and 2-deoxy-glucose (2-DG). Analyses in (**B**, **C**) were performed using measure #6 (blue arrow) for

basal glycolysis and measure #10 (red arrow) for maximal glycolytic capacity. (**D**) The mitochondrial respiration was established by sequential injections of oligomycin (Oligo), FCCP and the combination of rotenone (Rot) and antimycin A (AA). Analyses in (**E**, **F**) were performed using measure #3 (blue arrow) for basal mitochondrial respiration and measure #10 (red arrow) for maximal mitochondrial respiration. Data are presented as the mean ± SEM and displayed as percentages of controls (i.e., untreated condition) ($n = 8$; $N = 3$). * $p < 0.05$ by Student's *t*-test.

3.3.3.2. Cancer-Associated Fibroblasts Conditioned by Non-Invasive Bladder Cancer Cells Exhibit a Metabolic Switch, Characterized by a Decreased Mitochondrial Metabolism and Increased Glycolysis, Accentuated by BPA

HBFs were induced into cancer-associated fibroblasts (CAFs) using cell culture media conditioned by non-invasive bladder cancer cells (RT4). Compared to HBFs, CAFs conditioned by RT4 cell medium demonstrated generally increased glycolysis (**Figure 4.3A**) and significantly decreased mitochondrial respiration (**Figure 4.3D**), leading to a metabolic switch similar to the Warburg effect. When RT4 cells were chronically exposed to 10^{-8} M BPA, CAFs exhibited a significantly increased basal glycolytic capacity (**Figure 4.3B**) and maximal glycolytic capacity (**Figure 4.3C**). When HBFs were directly exposed to BPA during CAF induction, glycolysis was not affected, and the basal and maximal glycolytic capacities were similar to those of unexposed CAFs. Both the basal and maximal levels of mitochondrial respiration of CAFs remained unchanged when exposed directly or indirectly to BPA (**Figure 4.3E, F**). The metabolic switch observed in CAFs was therefore accentuated by chronic exposure of RT4 cells to physiological concentrations of BPA through increased glycolysis.

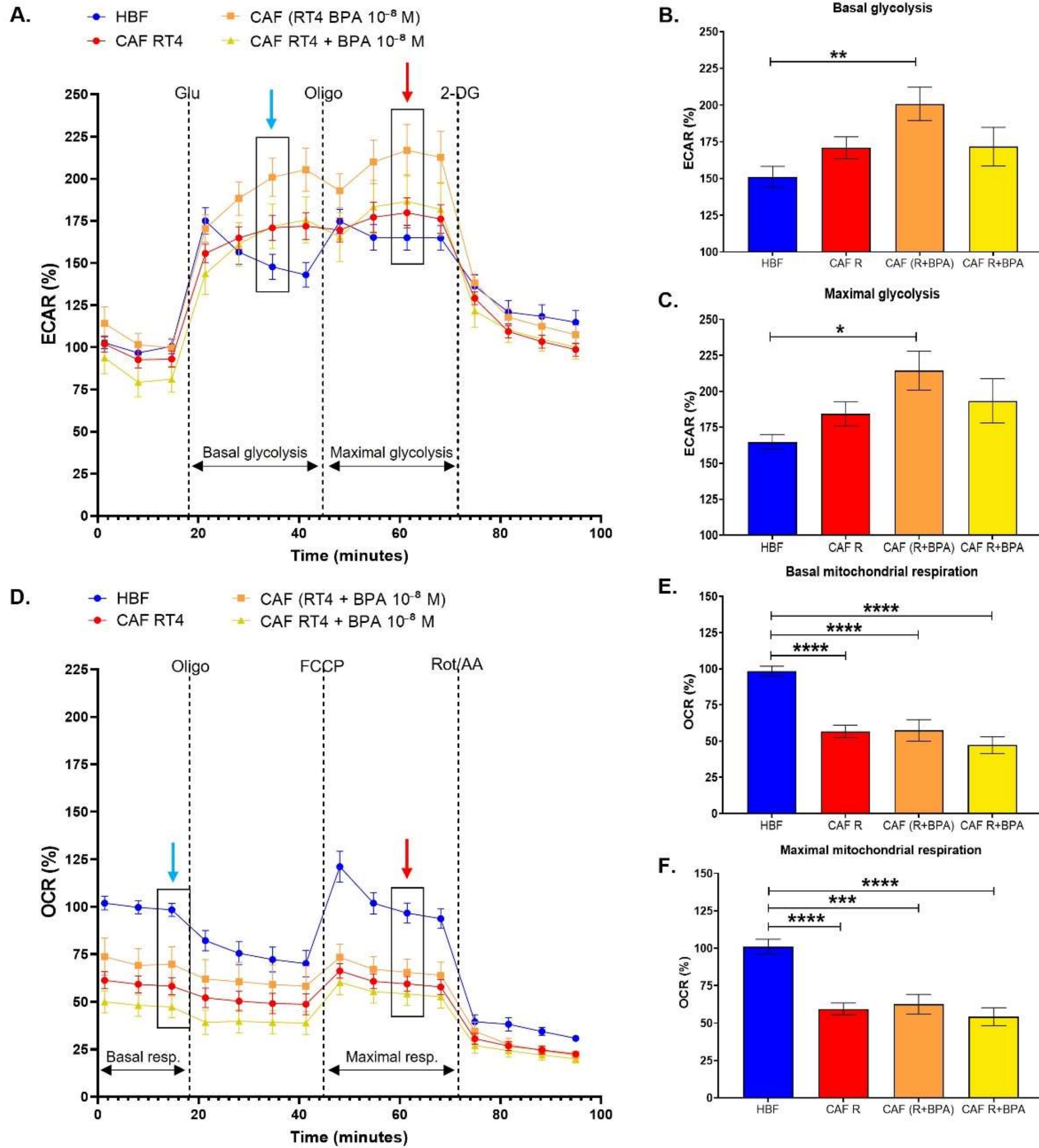

Figure 4.3. Cancer-associated fibroblasts conditioned by non-invasive bladder cancer cells exhibit a metabolic switch characterized by a decreased mitochondrial metabolism and increased glycolysis accentuated by BPA. (**A**–**C**) ECAR and (**D**–**F**) OCR were determined using the XFe96 Extracellular Flux Analyzer in cancer-associated fibroblasts (CAFs). Healthy human bladder fibroblasts (HBFs) were induced into CAFs over 8 days with conditioned media from non-invasive cancer cells (RT4) with exposure (direct or indirect) or not to 10^{-8} M BPA to establish (**B**) basal glycolysis, (**C**) maximal glycolytic capacity, (**E**) basal mitochondrial respiration and (**F**) maximal mitochondrial capacity. (**A**) The glycolytic metabolism and (**D**) mitochondrial respiration were established using sequential

injections, as in Figure 1. Analyses in (**B**, **C**) were performed using measure #6 (blue arrow) for basal glycolysis and measure #10 (red arrow) for maximal glycolytic capacity. Analyses in (**E**, **F**) were performed using measure #3 (blue arrow) for basal mitochondrial respiration and measure #10 (red arrow) for maximal mitochondrial respiration. Data are presented as the mean ± SEM and displayed as percentages of controls ($n = 8$; $N = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ by one-way ANOVA.

3.3.3.3. Cancer-Associated Fibroblasts Conditioned with Invasive Bladder Cancer Cells in the Presence of BPA Exhibit an Increased Glycolytic Metabolism

HBFs were induced into CAFs using cell culture media conditioned by invasive bladder cancer cells (T24). Compared to HBFs, CAFs conditioned by the culture medium of T24 cells did not exhibit a metabolic switch, unlike CAFs conditioned with RT4 culture medium. CAFs conditioned with T24 had similar glycolysis and mitochondrial respiration levels as HBFs (**Figure 4.4A, D**). The basal and maximal glycolytic capacities were unchanged by CAF induction (**Figure 4.4B, C**). However, CAFs exhibited significantly decreased basal mitochondrial respiration (**Figure 4.4E**), but the maximal mitochondrial respiration levels were similar to those of HBFs (**Figure 4.4F**). Therefore, CAF induction with T24-conditioned media did not seem to affect energy metabolism. On the other hand, when CAFs were directly or indirectly exposed to 10^{-8} M BPA, they exhibited an increased glycolytic metabolism. Chronic exposure to BPA resulted in a significantly increased basal (**Figure 4.4B**) and maximal glycolytic capacity (**Figure 4.4C**). Although direct exposure to BPA caused a significant increase in glycolysis, the chronic exposure of T24 to BPA (indirect exposure) resulted in a greater increase in both basal and maximal glycolytic capacities (**Figure 4.4B, C**). Chronic exposure to BPA did not seem to affect the mitochondrial respiration of CAFs conditioned with T24. Therefore, our results demonstrated that CAFs conditioned with T24 in the presence of BPA exhibit an increased glycolytic metabolism.

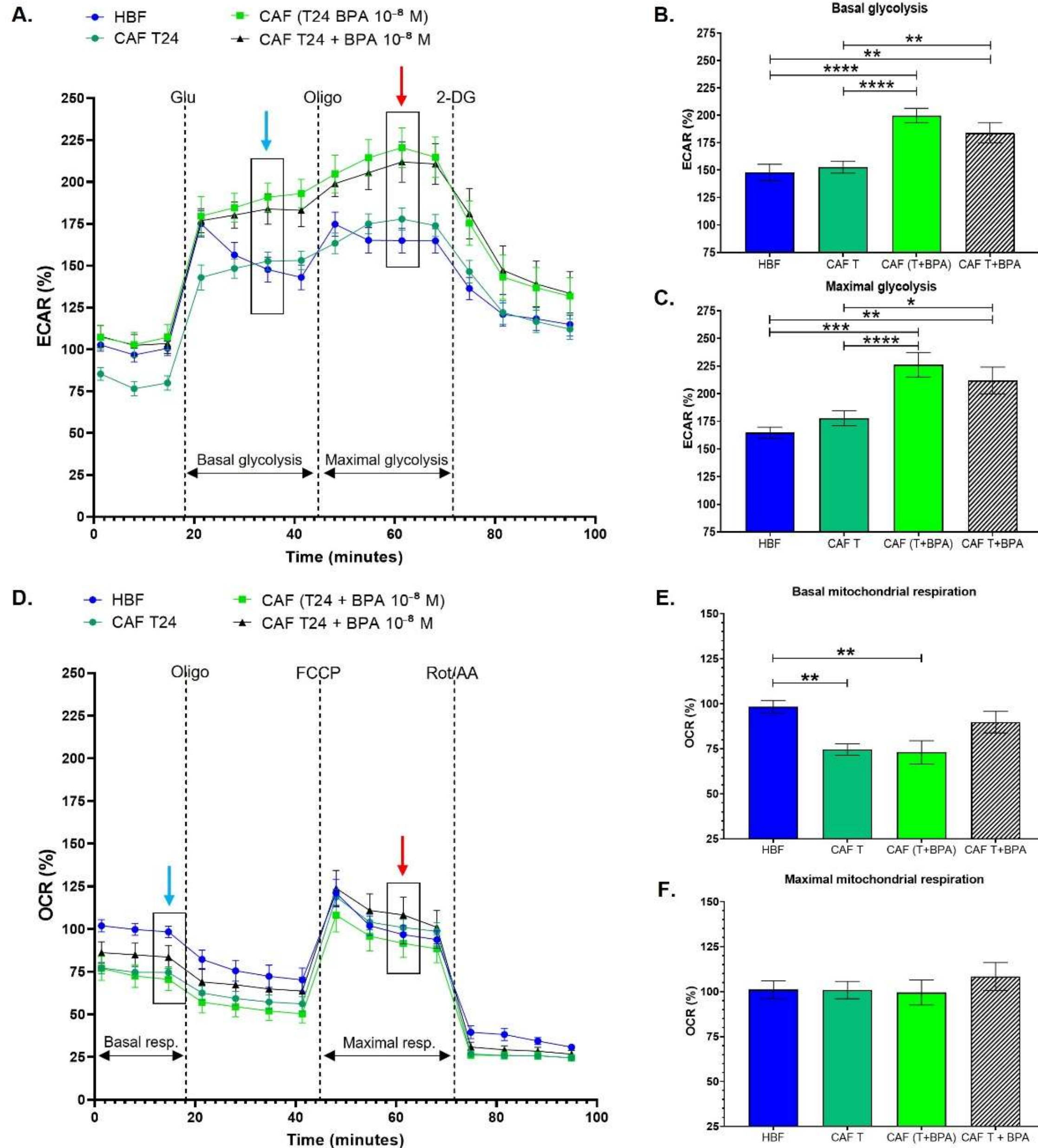

Figure 4.4. Cancer-associated fibroblasts conditioned with invasive bladder cancer cells in the presence of BPA exhibit an increased glycolytic metabolism. (**A**–**C**) ECAR and (**D**–**F**) OCR were determined using the XFe96 Extracellular Flux Analyzer in cancer-associated fibroblasts (CAFs). Healthy human bladder fibroblasts (HBFs) were induced into CAFs over 8 days with conditioned media from invasive cancer cells (T24) with exposure (direct or indirect) or not to 10^{-8} M BPA to establish (B) basal glycolysis, (**C**) maximal glycolytic capacity, (**E**) basal mitochondrial respiration and (**F**) maximal mitochondrial capacity. (**A**) The glycolytic metabolism and (**D**) mitochondrial respiration were established using sequential injections, as in Figure 1. Analyses in (**B**, **C**) were

performed using measure #6 (blue arrow) for basal glycolysis and measure #10 (red arrow) for maximal glycolytic capacity. Analyses in (**E**, **F**) were performed using measure #3 (blue arrow) for basal mitochondrial respiration and measure #10 (red arrow) for maximal mitochondrial respiration. Data are presented as the mean ± SEM and displayed as percentages of controls ($n = 8$; $N = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ by one-way ANOVA.

3.3.4. Discussion

As plastic products become more and more ubiquitous in our environment, studying the effects of the chemical compounds they can release, such as BPA, has become crucial. Growing evidence has shown the pro-tumorigenic effect of BPA [4–6] in many cancers, especially hormone-dependent cancers such as breast [5] and prostate cancer [7]. Although bladder tissue is not recognized as a hormone-sensitive tissue, studies have demonstrated the role of hormone receptors in bladder cancer initiation and progression [8,9], as well as their impact on treatment [20] and prognosis [21]. As the impact of BPA on bladder cancer has not yet been reported, the effects of BPA on the stromal environment of bladder cancer were studied through bladder fibroblasts and CAFs.

HBFs chronically exposed to physiological concentrations of BPA exhibited a decreased energy metabolism, characterized by a decreased glycolytic capacity and mitochondrial respiration. The consequences of cell detoxification could partly explain these observations. When cells are exposed to toxic substances, cells will detoxify themselves through ATP-binding cassette (ABC) transporters [22]. ABC transporters use the hydrolysis of adenosine triphosphate (ATP) to export these toxic molecules out of the cells [22], and biochemical studies revealed that up to two ATP molecules can be required to export one molecule of substrate [23]. Thus, the translocation of substrates at the expense of ATP hydrolysis catalyzed by ABC transporters leads to substantially high costs in energy consumption [24]. In this sense, bisphenols, including BPA, have been shown to interact with the ABC transporter breast cancer resistance protein (BCRP; coded by the gene *ABCG2*) [25]. Furthermore, studies have shown that BPA can inhibit metabolizing enzymes [26], thus affecting major metabolic pathways, such as mitochondrial respiration [27]. This inhibition

could lead to a decreased availability of substrates for metabolic pathways, resulting in a reduced energy metabolism. Our results have shown that HBFs have a decreased energy metabolism when exposed to BPA, suggesting that fibroblasts could be less functional. This could have substantial consequences on cellular functions, such as extracellular matrix (ECM) production. Fibroblasts are responsible for ECM production, which is crucial for tissue turnover and repair [28]. A decreased energy metabolism in HBFs caused by BPA exposure could affect wound healing and ECM turnover and compromise the bladder wall's capacity to repair damages or adequately sustain a differentiated urothelium with an optimal barrier function [29,30]. Urine contains toxic substances such as urea and carcinogens [31]. Studies have shown that the exposure of the urothelium to urinary carcinogens is linked to bladder cancer [32]. Therefore, a diminished wound healing capacity could result in the exposure of basal and intermediate urothelial cell layers to these substances and promote bladder cancer initiation through cell damage.

We also studied the impact of BPA on CAFs. First, the induction of HBFs into CAFs resulted in a metabolic switch characterized by an increased glycolytic capacity and decreased mitochondrial respiration, similar to the Warburg effect. Similar to our observation, it was reported that CAFs exhibit a metabolic switch where CAFs favor glycolysis to the detriment of mitochondrial respiration, even in the presence of oxygen [33]. Studies have suggested that TGF-β could have a role in this metabolic switch by increasing aerobic glycolysis, increasing oxidative stress, affecting the mitochondria's functioning and regulating certain enzymes [33–35]. Furthermore, studies by Ringuette-Goulet *et al.* have shown that invasive bladder cancer cells (T24) secrete more TGF-β than non-invasive bladder cancer cells (RT4) do [16]. Interestingly, TGF-β has been shown to activate the PI3K-Akt-mTOR pathway and promote the accumulation of activating transcription factor 4 (ATF4) in lung fibroblasts, leading to their metabolic reprogramming [36]. Another molecule that could be implicated is IL-6, which could impact the physiological activity of fibroblasts as well as potentialize the effects of TGF-β [37]. However, whether the alterations mediated by BPA in the energy metabolism of stromal cells are linked to TGF-β or IL-6 remains to be demonstrated.

CAF induction leads to increased glycolysis and, consequently, to increased production of lactate. An increased concentration of lactate in the ECM can have multiple consequences. Lactate inhibits immune cells, including monocytes involved in cancer cell elimination [38], which could promote cancer initiation and progression through the inhibition of the immune system [35]. Lactate directly leads to the acidification of the extracellular environment, promoting tumor invasion through a reorganization of the matrix [35,39]. Lactate also acts as a chemoattractant molecule and is used as a metabolite for cancer cells [33]. Thus, the increased lactate production following CAF induction allows cancer cells to increase their energy intake through the metabolites provided by CAFs [33]. The increased glycolytic capacity we observed, associated with an increased extracellular acidification rate, concurs with the increased lactate production described in the literature.

Our results on CAFs incubated with BPA showed that the metabolic switch observed in CAFs was accentuated through increased glycolysis when the cell culture medium was conditioned with non-invasive RT4 bladder cancer cells chronically exposed to physiological concentrations of BPA. Considering the impact of ECM acidification, the increased glycolysis resulting from BPA exposure could lead to higher lactate production. Therefore, by enhancing the glycolytic metabolism of CAFs, BPA could accentuate the consequences of lactate in the ECM, resulting in a more important inhibition of the immune cells, a higher energy intake for cancer cells and a critical matrix remodeling. By affecting the glycolytic metabolism of CAFs, BPA could promote the invasion of non-invasive cancer cells through alteration of the ECM and therefore support bladder cancer progression through stromal cells.

Compared to CAFs conditioned with medium from non-invasive RT4 cells, CAFs conditioned with medium from invasive T24 cells did not exhibit a metabolic switch similar to the Warburg effect. The absence of a metabolic switch for CAFs conditioned with medium from T24 cells could partially be explained by the fact that T24 cells are already invasive cancer cells and could therefore be less likely to need CAFs' contribution to allow cell invasion. Furthermore, the limited changes in the metabolism of CAFs conditioned with medium from T24 cells could be explained by the simplicity of the 2D model, which does not include the interactions between cells and ECM proteins. Certain stimuli, such as the

compression induced by the tumor mass on the stroma, will induce a metabolic change [40]. However, it is important to note that BPA by itself can induce similar metabolic changes. The presence of BPA in a 3D model could potentially accentuate these changes.

Although chronic exposure to BPA did not affect the mitochondrial respiration of CAFs conditioned by invasive T24 bladder cancer cells, our results demonstrated that BPA induced an increased glycolytic metabolism. As explained earlier, increased glycolysis leads to an augmentation of lactate production and acidification of the ECM. Even if T24 cells are invasive cancer cells, BPA could facilitate cancer cell invasion by altering the glycolytic metabolism of CAFs and lead to metastasis generation. BPA could therefore promote invasion of bladder cancer cells.

The use of an in vitro 2D cell culture is one of the limitations of this study. The use of a 3D bladder cancer model [18] could allow a better physiological representation of the impact of BPA on the stromal environment. A 3D model could allow us to further study the impacts of chronic exposure to BPA on ECM production by bladder fibroblasts as well as ECM alteration through CAF induction and BPA exposure. Another limitation of this study is the inability to obtain a perfectly bisphenol-free control in experimental settings [41]. In fact, external bisphenol contamination is difficult to avoid because of the ubiquity of bisphenol-based plastics in laboratory equipment and scientific instruments and the leaching properties of these compounds [42]. Thus, the bisphenol concentrations we added in our experiments might be slightly underestimated, but we nonetheless observed significant differences between the BPA condition and the negative controls. The impact of BPA alternatives, such as bisphenol S (BPS), on bladder cancer would also be of relevance. BPS is now used in a wide variety of plastic products to progressively replace BPA [43]. Although BPS is supposed to be a safer alternative, growing evidence shows that BPS has endocrine-disrupting capabilities similar to or even stronger than BPA [43,44] and could also promote cancer progression [45].

3.3.5. Conclusions

Our study has brought a new insight on the potential impact of BPA, an environmental pollutant, on bladder cancer progression with the evaluation of the impact of BPA on the stromal environment of bladder cancer. Using bladder fibroblasts and CAFs, we demonstrated that the energy metabolism of HBFs is negatively affected by BPA, while the exposure of CAFs to BPA could promote bladder cancer progression and invasion through an altered metabolism. The observed effect of BPA on HBFs and CAFs could partially explain the physiopathology of bladder cancer. In fact, bladder cancer is characterized by elevated levels of recurrence where non-invasive bladder cancer can evolve into invasive bladder cancer. Therefore, ubiquitous and continuous exposure to endocrine disruptors, such as BPA, could have an impact at the clinical level and affect patients' prognosis.

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Discussion

Au cours de mon projet de maîtrise, j'ai cherché à déterminer les effets d'une exposition chronique aux bisphénols, plus précisément le BPA et le BPS, sur les cellules urothéliales saines, les cellules cancéreuses de vessie non-invasives et invasives, les fibroblastes vésicaux et les CAF. Afin de bien caractériser l'impact de ces composés synthétiques sur le cancer de la vessie, nous avons évalué le métabolisme énergétique, la prolifération et la migration cellulaire.

Le premier chapitre de ce mémoire présentait une revue exhaustive de la littérature afin d'établir les potentiels liens entre les perturbateurs endocriniens et les cancers urologiques. À la suite de cet article de revue, nous avons observé qu'il n'existait aucune recherche sur les bisphénols et le cancer de la vessie. En fait, de plus en plus d'études abordent l'impact d'une exposition au BPA ou au BPS sur le cancer de la prostate, mais leurs conséquences sur l'urothélium et le microenvironnement tumoral vésical n'étaient pas établies. Notre revue des études effectuées nous a permis d'identifier les paramètres essentiels à évaluer, afin d'éviter la redondance et les expériences non pertinentes. Ces constatations nous ont ainsi mené à étudier l'influence des bisphénols sur le comportement des cellules vésicales saines et cancéreuses via la bioénergétique, la prolifération et la migration cellulaire.

Le deuxième chapitre de ce mémoire présentait nos résultats sur l'impact du BPA et du BPS sur la bioénergétique et le comportement des cellules urothéliales et cancéreuses de vessie. Avant d'établir les conséquences d'une exposition chronique aux bisphénols, nous avons comparé le métabolisme énergétique de deux populations de cellules urothéliales saines avec des cellules cancéreuses RT4 non-invasives et T24 invasives de vessie. Tout d'abord, nos résultats ont démontré que les deux populations de cellules urothéliales présentaient des métabolismes énergétiques similaires. Ensuite, les cellules cancéreuses non-invasives RT4 ont un métabolisme glycolytique de base et une capacité mitochondriale plus élevés que les cellules urothéliales. Finalement, les cellules cancéreuses invasives T24 présentent une capacité glycolytique accentuée, comparativement aux autres lignées cellulaires. Ces résultats étaient attendus, puisque les cellules cancéreuses sont caractérisées par une reprogrammation métabolique, caractérisée par une augmentation de la glycolyse au

détriment de la respiration mitochondriale, malgré la présence d'oxygène et de mitochondries fonctionnelles (Liberti & Locasale, 2016). Il s'agit de l'effet de Warburg. Cette adaptation métabolique permet de supporter la croissance et la prolifération importante des cellules cancéreuses (Liberti & Locasale, 2016). Ce phénomène provoque une accumulation de lactate dans le milieu extracellulaire, ce qui induit une acidification de la matrice. Ceci favorise l'inhibition des cellules immunitaires et permet un remodelage de la matrice afin de promouvoir l'invasion tumorale (Goetze et al., 2011; Niu et al., 2021).

À la suite de la caractérisation de la bioénergétique de nos lignées cellulaires, nous avons étudié les effets d'une exposition chronique aux bisphénols sur le métabolisme énergétique, la prolifération, la migration et l'expression d'α-SMA de ces cellules. Tout d'abord, nos résultats ont démontré qu'une exposition à des concentrations physiologiques de BPA ou de BPS augmente la prolifération des cellules urothéliales saines, mais diminue leur migration, ce qui pourrait mener à de l'hyperplasie des cellules épithéliales et une altération des capacités de réparation de l'urothélium (Schultz, Chin, Moldawer, & Diegelmann, 2011). Ensuite, nous avons démontré qu'une exposition chronique au BPA ou au BPS augmentait le métabolisme énergétique et l'activité physiologique des cellules cancéreuses. Un élément important à retenir est l'augmentation de la bioénergétique, de la prolifération et de l'expression d'α-SMA chez les cellules cancéreuses non-invasives RT4 à la suite d'une exposition aux bisphénols. L'α-SMA peut être utilisé comme marqueur pour l'EMT (Anggorowati et al., 2017). Une expression élevée d'α-SMA chez les cellules cancéreuses est habituellement associée à un phénotype invasif et à une capacité accrue de générer des métastases (Anggorowati et al., 2017). En effet, un niveau d'expression élevé d'α-SMA est associé à un mauvais pronostic dans les cancers du sein et du poumon (Jeon et al., 2017; Lee et al., 2013). À la suite d'une exposition au BPA, les cellules cancéreuses non-invasives RT4 expriment plus fortement l'α-SMA, ce qui suggère une augmentation de l'agressivité, et donc un phénotype plus invasif. Ainsi, ces résultats suggèrent que le BPA et le BPS pourraient favoriser la progression d'un cancer de vessie non-invasif en cancer invasif. En conséquence, l'exposition chronique aux bisphénols pourrait avoir un impact clinique important dans la récidive, la progression et le pronostic des patients atteints d'un cancer de la vessie, même superficiel.

Nos résultats obtenus au niveau de l'urothélium vésical nous ont mené à nous questionner sur l'impact du BPA sur les cellules stromales. Ainsi, nous avons évalué les effets d'une exposition chronique au BPA sur les fibroblastes vésicaux et les CAF. Les résultats de cette étude ont été présentés dans le troisième chapitre de ce mémoire. Tout d'abord, nos résultats ont démontré que les fibroblastes vésicaux exposés chroniquement à des concentrations physiologiques de BPA présentaient un métabolisme énergétique diminué. Ce phénomène pourrait en partie être attribuable à la détoxification des cellules stromales via les transporteurs ABC. Ces transporteurs utilisent de grandes quantités d'ATP pour exporter les molécules toxiques hors des cellules (Beis, 2015). Puisque le BPA peut interagir avec ces transporteurs (Engdahl et al., 2021), ce composé pourrait provoquer l'épuisement énergétique des cellules. De plus, le BPA peut inhiber des enzymes liées au métabolisme, dont certaines enzymes associées à la respiration mitochondriale (Khan et al., 2016; Quesnot et al., 2014). Le métabolisme énergétique diminué des fibroblastes vésicaux exposés au BPA suggère que ces cellules semblent moins actives, ce qui pourrait entraîner des conséquences sur la production de la matrice extracellulaire. La production d'une matrice adéquate est essentielle pour assurer le renouvellement et la réparation du stroma (Bainbridge, 2013). Ainsi, les effets du BPA observés sur les fibroblastes vésicaux pourraient altérer la réparation du tissu vésical, et empêcher une différenciation adéquate de l'urothélium (Bouhout et al., 2016). Ceci pourrait, entre autres, affecter la fonction barrière de l'urothélium et provoquer des fuites internes d'urine dans l'organisme. Les tissus sains environnants peuvent ainsi être exposés aux carcinogènes retrouvés dans l'urine (Hecht, 2002), ce qui peut promouvoir le développement tumoral (Silverman et al., 2008).

Nos résultats ont ensuite démontré que l'induction des fibroblastes vésicaux sains en CAF par l'intermédiaire de milieu conditionné par des cellules cancéreuses non-invasives RT4 induisait une reprogrammation métabolique. Ce phénomène observé chez les CAF était similaire à l'effet de Warburg. Les fibroblastes vésicaux ne présentaient pas d'effet de Warburg, puisque ce sont des cellules stromales saines. Tout comme chez les cellules cancéreuses, l'augmentation de la glycolyse chez les CAF résulte en une accumulation de lactate dans l'espace extracellulaire. Dans le microenvironnement tumoral, cette acidification inhibe les cellules immunitaires et favorise l'invasion tumorale via un remodelage de la

matrice (Goetze et al., 2011; Niu et al., 2021). De plus, le lactate produit en quantité excessive par les CAF peut servir de substrat énergétique pour les cellules cancéreuses, ce qui permet de supporter les besoins énergétiques accentués de ces dernières (Pavlides et al., 2009). À la suite d'une exposition prolongée au BPA, les CAF présentaient une reprogrammation métabolique davantage accentuée, caractérisée par une augmentation de la glycolyse. Ainsi, nos résultats suggèrent qu'une exposition au BPA via l'urine pourrait mener à une acidification plus importante du milieu extracellulaire par les CAF, augmentant le remodelage de la matrice, inhibant davantage les cellules immunitaires et permettant un apport en énergie plus important au niveau de la tumeur. Toutefois, la détoxification et l'exportation des bisphénols par les transporteurs ABC ne peuvent pas être exclues chez les cellules cancéreuses. Ce phénomène pourrait être dissimulé par l'augmentation des capacités métaboliques des cellules cancéreuses par les bisphénols. Les effets observés du BPA sur les fibroblastes vésicaux et les CAF pourraient, en partie, expliquer la récidive accrue du CaV (Metts et al., 2000). En effet, en affectant le métabolisme des cellules stromales, le BPA pourrait favoriser la progression des cancers non-invasifs en cancers invasifs. L'exposition chronique aux bisphénols pourrait donc avoir un impact important au niveau clinique.

Les résultats de ce projet de recherche ont ainsi démontré les effets potentiels d'une exposition chronique aux bisphénols sur l'initiation et la progression du cancer de la vessie. En affectant à la fois les cellules urothéliales saines et cancéreuses, ainsi que les cellules stromales (fibroblastes vésicaux et CAF), les bisphénols pourraient favoriser la transition des cancers de vessie non-invasifs en cancer invasifs. L'exposition environnementale de l'être humain aux plastiques est donc un aspect qui devrait être étudié davantage, puisque celle-ci pourrait jouer un rôle significatif dans la récidive et l'évolution des cancers de la vessie.

L'omniprésence des plastiques dans le domaine de la recherche fait en sorte qu'il est difficile d'assurer une condition contrôle totalement dépourvue de bisphénols (vom Saal & Welshons, 2014). Puisqu'il n'est pas possible de prédire les quantités de bisphénols libérés par l'équipement de laboratoire, tels que les tubes et les flacons de culture cellulaires faits de plastiques, les concentrations en bisphénols utilisées dans nos expériences pourraient être légèrement sous-estimées (Vandenberg, Hauser, Marcus, Olea, & Welshons, 2007). Cet

aspect constitue une des limitations de ce projet de recherche. Toutefois, nos résultats démontrent tout de même des différences significatives entre les contrôles négatifs et les échantillons conditionnés au BPA ou au BPS. De plus, le fait d'avoir effectué nos expériences sur monocouche 2D correspond bien sûr à une autre limitation de ce projet de recherche. L'utilisation d'un modèle 3D de cancer de la vessie reconstruit par génie tissulaire (Ringuette Goulet et al., 2017) correspond à un modèle plus physiologique qui permettrait une meilleure représentation des impacts du BPA et du BPS sur le microenvironnement tumoral du cancer de la vessie.

L'évaluation de l'impact des bisphénols sur des modèles 3D est une des perspectives de ce projet de recherche. En fait, il serait possible d'augmenter graduellement la complexité des modèles utilisés afin de caractériser de manière extensive les conséquences d'une exposition aux bisphénols. Tout d'abord, il serait possible d'utiliser des sphéroïdes de cellules cancéreuses, qui représentent un modèle plus représentatif du cancer qu'une monocouche de cellules cancéreuses. Ensuite, ces sphéroïdes pourraient être intégrés dans un modèle 3D simple, soit le gel de collagène acellularisé ou cellularisé de fibroblastes sains ou de CAF, puis dans un modèle 3D complexe, soit un tissu vésical reconstruit par génie tissulaire. Le séquençage de l'ARN des cellules exposées aux bisphénols est une perspective intéressante qui permettrait d'établir les cibles au niveau moléculaire. Ceci permettrait d'identifier les voies de signalisation impliquées dans l'altération du métabolisme énergétique et de l'activité physiologique des cellules par le BPA et le BPS. Cette expérience fournirait les informations nécessaires afin de caractériser de manière exhaustive les voies moléculaires affectées par les bisphénols dans le cancer de la vessie. De plus, l'étude de l'impact des métabolites des bisphénols sur le cancer de la vessie serait un élément pertinent. En effet, une proportion importante des bisphénols ingérés est métabolisée et excrétée via l'urine (Genuis et al., 2012). Des études ont démontré que certains de ces métabolites pouvaient demeurer actifs dans l'organisme (Boucher, Boudreau, Ahmed, & Atlas, 2015), suggérant de ces derniers pourraient aussi avoir un impact sur le cancer de la vessie. Nous sommes présentement en cours de rédaction d'un article portant sur les effets des métabolites glucuronidés sur les cellules urothéliales et cancéreuses de vessie. Nous avons vérifié l'impact d'une exposition chronique aux métabolites glucuronidés du BPA et du BPS sur le métabolisme énergétique,

la prolifération et la migration des cellules urothéliales saines, des cellules cancéreuses RT4 et T24. Nos résultats démontrent que ces métabolites semblent avoir des effets similaires aux molécules d'origine. En effet, l'exposition chronique aux métabolites glucuronidés diminue le métabolisme énergétique et l'activité physiologique des cellules urothéliales saines, tandis que ces paramètres sont augmentés chez les cellules cancéreuses. Ainsi, même sous forme métabolisée, les bisphénols semblent avoir un impact sur le développement du cancer de la vessie.

Conclusion

Ce projet de recherche avait pour objectif général de déterminer l'impact d'une exposition aux bisphénols sur l'initiation et la progression du cancer de la vessie. Notre hypothèse de départ était que l'exposition chronique à des concentrations physiologiques de BPA ou de BPS allait altérer le métabolisme énergétique et l'activité physiologique des cellules urothéliales saines, des cellules cancéreuses de vessie, des fibroblastes vésicaux et des fibroblastes associés au cancer, afin de favoriser le développement et la progression du cancer de la vessie. Les résultats présentés dans les chapitres deux et trois de ce mémoire permettent de confirmer cette hypothèse. En effet, nos résultats obtenus chez les cellules urothéliales saines et les cellules cancéreuses de vessie suggèrent qu'une exposition chronique au BPA ou au BPS pourrait favoriser la progression des cancers non-invasifs en cancers invasifs. De plus, nos résultats obtenus chez les fibroblastes vésicaux sains et les CAF ont démontré que le BPA pourrait altérer la production de matrice et ainsi favoriser l'invasion tumorale.

Puisqu'aucune étude n'avait précédemment étudié les possibles liens entre l'exposition aux bisphénols et le cancer de la vessie, nos travaux ont permis d'apporter des informations innovantes et significatives. Ainsi, nous avons exploré l'impact de perturbateurs endocriniens sur ce cancer urologique. Au cours de nos recherches, nous avons démontré que l'exposition à long terme au BPA ou au BPS pourrait avoir un impact sur le développement du cancer de la vessie. Ces travaux nous ont permis d'avoir une meilleure compréhension des potentielles conséquences de l'exposition chronique à la pollution par les plastiques sur le développement et la progression du cancer de la vessie. Davantage d'études seront nécessaires afin d'identifier les conséquences cliniques pouvant découler d'une telle exposition continue.

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Annexe A : Articles supplémentaires publiés

En plus des articles présentés dans ce mémoire, j'ai participé à la rédaction de deux autres articles scientifiques portant sur des sujets connexes à mon projet de recherche. Le premier article scientifique, publié en novembre 2021 dans le journal *Bioengineering*, porte sur l'impact de l'inactivation des sérums utilisés en culture cellulaire sur différentes populations de fibroblastes, soit des fibroblastes vésicaux, dermiques et vaginaux (**Annexe A.I**). Le deuxième article scientifique, publié en mai 2022 dans le journal *Oncology Letters*, porte sur l'impact de l'hypoxie sur le profil des cellules cancéreuses de vessie (**Annexe A.II**). Les informations spécifiques et les résumés en anglais de ces articles sont présentés ci-dessous.

Annexe A.I : Heat-Inactivation of Fetal and Newborn Sera Did Not Impair the Expansion and Scaffold Engineering Potentials of Fibroblasts

Pellerin, F. A., Caneparo, C., Pellerin, È., Chabaud, S., Pelletier, M., & Bolduc, S. (2021). Heat-Inactivation of Fetal and Newborn Sera Did Not Impair the Expansion and Scaffold Engineering Potentials of Fibroblasts. *Bioengineering (Basel, Switzerland)*, *8*(11), 184. https://doi.org/10.3390/bioengineering8110184

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Rôle dans la préparation de l'article : acquisition, analyse et interprétation des données, révision finale de l'article

Coauteurs : Félix-Antoine Pellerin, Christophe Caneparo, Dr Stéphane Chabaud, Dr Martin Pelletier et Dr Stéphane Bolduc

Abstract: Heat inactivation of bovine sera is routinely performed in cell culture laboratories. Nevertheless, it remains debatable whether it is still necessary due to the improvement of the production process of bovine sera. Do the benefits balance the loss of many proteins, such as hormones and growth factors, that are very useful for cell culture? This is even truer in the case of tissue engineering, the processes of which is often very demanding. This balance is examined here, from nine populations of fibroblasts originating from three different organs, by comparing the capacity of adhesion and proliferation of cells, their metabolism, and the capacity to produce the stroma; their histological appearance, thickness, and mechanical properties were also evaluated. Overall, serum inactivation does not appear to provide a significant benefit.

Annexe A.II : **Bladder cancer cell lines adapt their aggressiveness profile to oxygen tension**

Chabaud*, S., Pellerin*, È., Caneparo, C., Ringuette-Goulet, C., Pouliot, F., & Bolduc, S. (2022). Bladder cancer cell lines adapt their aggressiveness profile to oxygen tension. *Oncology letters*, *24*(1), 220. https://doi.org/10.3892/ol.2022.13341

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Rôle dans la préparation de l'article : acquisition, analyse et interprétation des données, révision finale de l'article

Coauteurs : Dr Stéphane Chabaud, Christophe Caneparo, Cassandra Ringuette-Goulet, Dr Frédéric Pouliot et Dr Stéphane Bolduc

Abstract: During the process of tumor growth, cancer cells will be subjected to intermittent hypoxia. This results from the delay in the development of the vascular network in relation to the proliferation of cancer cells. The hypoxic nature of a tumor has been demonstrated as a negative factor for patient survival. To evaluate the impact of hypoxia on the survival and migration properties of low and high-grade bladder cancer cell lines, two low-grade (MGHU-3 and SW-780) and two high-grade (SW-1710 and T24) bladder cancer cell lines were cultured in normoxic (20% O_2) or hypoxic atmospheric conditions (2% O_2). The response of bladder cancer cell lines to hypoxic atmospheric cell culture conditions was examined under several parameters, including epithelial-mesenchymal transition, doubling time and metabolic activities, thrombospondin-1 expression, whole Matrix Metallo-Proteinase activity, migration and resistance to oxidative stress. The low-grade cell line response to hypoxia was heterogeneous even if it tended to adopt a more aggressive profile. Hypoxia enhanced migration and pro-survival properties of MGHU-3 cells, whereas these features were reduced for the SW-780 cell line cultured under low oxygen tension. The responses of tested high-grade cell lines were more homogeneous and tended to adopt a less aggressive profile. Hypoxia drastically changed some of the bladder cancer cell line properties, for example matrix metalloproteinases expression for all cancer cells but also switch in glycolytic metabolism of low grade cancer cells. Overall, studying bladder cancer cells in hypoxic environments are relevant for the translation from *in vitro* findings to *in vivo* context.