UNIVERSIDADE FEDERAL DE UBERLÂNDIA
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DAYANNE SILVA BORGES

AVALIAÇÃO DAS ALTERAÇÕES CELULARES E MOLECULARES MEDIADAS POR PESTICIDAS EM CÉLULAS PROSTÁTICAS HUMANAS

PATOS DE MINAS – MG
DEZEMBRO DE 2022

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Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Biotecnologia como requisito parcial para obtenção do título de Mestre em Biotecnologia.

Orientadora: Profa. Dra. Thaise Gonçalves de Araújo

Co-orientador: Dr. Raoni Pais Siqueira

PATOS DE MINAS – MG

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Data:	19/12/2022	Hora de início:	14:10	Hora de encerramento:	16:40
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Reuniu-se, na sala 104 da Major Jerônimo, no Campus Patos de Minas, da Universidade Federal de Uberlândia e, de forma remota, por meio de videoconferência a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Biotecnologia, assim composta: Professoras Doutoras: Cristina Ribas Fürstenau (UFABC - videoconferência), Joyce Ferreira da Costa Guerra (UFU) e Thaise Gonçalves de Araújo (UFU), orientadora da candidata.

Iniciando os trabalhos a presidente da mesa, Profa. Dra. Thaise Gonçalves de Araújo (UFU), apresentou a Comissão Examinadora e a candidata, agradeceu a presença do público, e concedeu à Discente a palavra para a exposição do seu trabalho. A duração da apresentação da Discente e o tempo de arguição e resposta foram conforme as normas do Programa.

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O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU.

Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

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Aprovado em ___/___/___

BANCA EXAMINADORA

_____

Prof(a). Dra. Thaise Gonçalves de Araújo - UFU

_____

Prof(a). Dra. Cristina Ribas Furstenau - UFABC

_____

Prof(a). Dra. Joyce Ferreira da Costa Guerra - UFU

PATOS DE MINAS – MG

2022

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RESUMO

A economia do Brasil é baseada na produção agrícola sendo um dos maiores exportadores de *commodities* mundialmente. Para isso, o país aumentou a qualidade e a produtividade de suas culturas, tornando-se um importante consumidor de pesticidas, principalmente de Glifosato (GLI) e seu principal subproduto, o Ácido Aminometilfosfônico (AMPA). O consumo exacerbado e/ou frequente dessas substâncias já foi relacionado com o desenvolvimento de diversas doenças. Diante disso, o presente estudo objetivou compreender a modulação de eventos relacionados à tumorigênese prostática mediada pelos herbicidas GLI e AMPA. A viabilidade celular foi inicialmente avaliada pelo método MTT em células prostáticas não-tumorigênicas (PNT2) e tumorais LNCaP (hormônio-dependente) e PC-3 (hormônio-independente). Em células PNT2, a expressão relativa dos transcritos foi analisada pelo método qPCR e a capacidade proliferativa foi verificada por meio do ensaio clonogênico. Foi também determinada a expressão da Glutationa S-transferase Mu3 (GSTM3), a atividade enzimática de GSTs e a oxidação proteica. Os resultados mostram que GLI e AMPA foram citotóxicos após 48 horas de tratamento e apenas em concentrações mais altas, principalmente na linhagem PNT2. Em contrapartida, esses compostos aumentaram a formação de colônias nestas células, especialmente nas concentrações abaixo dos valores de IC_{50} (GLI a 5 mM e AMPA 10 mM). Nessa condição, houve um aumento nos níveis transcricionais do gene *GSTM3*, assim como em sua expressão proteica. No entanto, a atividade enzimática de GST foi significativamente diminuída, com um aumento no estresse oxidativo. Portanto, GLI e AMPA são capazes de conduzir alterações moleculares em células PNT2, especialmente relacionadas ao estresse oxidativo, mesmo em concentrações não citotóxicas. Estudos adicionais são necessários para melhor compreender os efeitos desses pesticidas, especialmente em concentrações residuais.

Palavras-chave: Próstata. Herbicidas. GSTM3. Transcritos. Estresse Oxidativo.

ABSTRACT

Brazil's economy is based on agricultural production, being one of the largest exporters of commodities worldwide. To this end, the country increased the quality and productivity of its crops, becoming an important consumer of pesticides, mainly Glyphosate (GLI) and its main by-product, Aminomethylphosphonic acid (AMPA). The exacerbated and/or frequent consumption of these substances has already been related to the development of several diseases.Therefore, the present study aimed to understand the modulation of events related to prostatic tumorigenesis mediated by the herbicides Glyphosate (GLI) and its main by-product, Aminomethylphosphonic acid (AMPA). Cell viability was initially evaluated by the MTT method in non-tumorigenic prostatic cells (PNT2), LNCaP (hormone-dependent PCa), and PC-3 (hormone-independent PCa). In PNT2 cells, the relative expression of the transcripts was analyzed by the qPCR method, and the proliferative capacity was verified by clonogenic assay. The expression of Glutathione S-transferase Mu3 (GSTM3), the enzymatic activity of GSTs, and protein oxidation were also determined. The results show that GLI and AMPA were cytotoxic after 48 hours of treatment and only at higher concentrations, mainly in the PNT2 lineage. On the other hand, these compounds increased colony formation, especially at concentrations below IC50 values (5 mM GLI and 10 mM AMPA). In this condition, there was an increase in transcriptional levels of the *GSTM3* gene, as well as in its protein expression. However, GST enzymatic activity was significantly decreased, with an increase in oxidative stress. Therefore, GLI and AMPA can drive molecular changes in PNT2 cells, especially related to oxidative stress, even at non-cytotoxic concentrations. Additional studies are needed to better understand the effects of these herbicides, especially at residual concentrations.

Keywords: *Prostate. Herbicides. GSTM3. Transcripts. Oxidative stress.*

Lista de abreviaturas e siglas

ADT: Terapia de Privação Androgênica

AMPA: Ácido Aminometilfosfônico

ANXA1: Anexina A1

AR: Receptor de Andrógeno

ATZ: Atrazina

Bcl-2: Proteína 2 do Linfoma de Células B

BK: Vírus BK

BRCA1: Breast Cancer 1

BRCA 2: Breast Cancer 2

B2M: Beta-2-microglobulina

CaP: Câncer de Próstata

CDNB: 1-chloro-2,4-dinitrobenzene

CDH1: Caderina 1

CK: Citoqueratina

CK5: Citoqueratina 5

CK8: Citoqueratina 8

CK14: Citoqueratina 14

CK18: Citoqueratina 18

CM: Câncer de Mama

C-N: Ligação carbono-nitrogênio

C-P: Ligação fósforo-carbono

CPRC: Câncer de Próstata resistente à castração

DDE: Diclorodifenildicloroetano

DDT: Dicloro-Difenil-Tricloroetano ácido

DHT: Diidrotestosterona

DL50: Dose letal para 50% da população teste

DMSO: Dimetilsulfóxido

DNA: Ácido desoxirribonucleico

DNP: 2,4-denitrophenylhydrazone

DP: Doença de Parkinson

EBV: Vírus Epstein-Barr

EDTA: Ácido etilenodiamino tetra-acético

EG: Escore de Gleason

EMT: transição Epitelial-mesenquimal

EO: Estresse Oxidativo

EPHB2: Receptor 2 da Efrina tipo B

EPIs: Equipamentos de Proteção Individual

EPSPS: Enzima 5-enolpiruvilshiquimato-3-fosfato sintase

FDA: *Food and Drug Administration*

FBS: Soro fetal bovino

FSH: Hormônio Folículo Estimulante

GAS5: Gene 5 Específico para parada do crescimento

GLI: Glifosato

GSH: Glutationa reduzida

GST: Glutationa S-transferase

GSTM3: Glutationa S-transferase Mu 3

HBG: Herbicidas à Base de Glifosato

HCMV: Citomegalovírus Humano

hK3: Calicreína Humana 3

HPB: Hiperplasia Prostática Benigna

HPV: Papilomavírus humano

IARC*: International Agency for Research on Cancer*

IBAMA: Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis

IC50= Concentração inibitória média

IL6: Interleucina 6

IL8: Interleucina 8

JC: Vírus John Cunningham

kDa*:* Quilodalton

KLK3: gene codificante da calicreína humana 3 ou Antígeno Prostático Específico

LH: Hormônio Luteinizante

LHRH: Receptor do hormônio liberador do hormônio luteinizante

lncRNA: RNA Longo Não Codificante

MTT: 3- (4,5-dimetiltiazol-2yl) -2,5-difenil brometo de tetrazolina

NETO2: Neuropilina e Proteína Tipo Lollóide 2

p63: Proteína 63

PBS: Tampão fosfato-salino

PAP: Fosfatase Ácida Prostática

PPA: Potencial de Periculosidade Ambiental

PR: prostatectomia radical

PRUNE2: Prune Homólogo 2 com domínio BCH

PSA: Antígeno Prostático Específico

OMS: Organização Mundial da Saúde

OS: Estresse oxidativo

RNA: Ácido ribonucleico

ROS: Espécies reativas de oxigênio

RPMI-1640: *Roswell Park Memorial Institute medium*

SNC: Sistema Nervoso Central

SNP: Sistema Nervoso Periférico

SNPs: Polimorfismos de Nucleotídeo Único

SV40: Vírus Vacuolante Símio 40

TEA: Transtorno do Espectro do Autismo

TFA: Ácido trifluoroacético

TGFβ1: Fator de Crescimento Transformador Beta 1

TNM: *Tumor Node Metastasis*

TR: Toque retal

VIM: Vimentina

qPCR: PCR em tempo real

ZC: Zona Central

ZP: Zona Periférica

ZT: Zona de Transição

2,4-D: 2,4-diclorofenoxiacético

SUMÁRIO

CAPÍTULO 1

1. INTRODUÇÃO

1.1 Problema

O câncer é um problema de saúde pública devido ao seu caráter epidêmico, com altas taxas de morbidade e mortalidade (ALVES; MAGALHÃES; COELHO, 2017). Somente em 2018, aproximadamente 9,6 milhões de pessoas morreram pela doença sendo 6,7 milhões pertencentes a países de baixa e média rendas (WHO, 2020). Para o ano 2030, segundo os dados divulgados pela Organização Mundial da Saúde (OMS), esperam-se 27 milhões de novos casos, 17 milhões de óbitos e um total de 75 milhões de pacientes oncológicos (BRASIL, 2011). Para 2020 ocorreram, nos Estados Unidos, em torno de 1,8 milhões de novos diagnósticos da doença, com 606 mil mortes. Os dados são ainda mais alarmantes para os cânceres de pulmão, com 228 mil novos casos e 112 mil mortes, e próstata com 191 mil novos casos e 33 mil mortes (SIEGEL; MILLER; JEMAL, 2020).

No Brasil, o Câncer de Próstata (CaP) é a segunda neoplasia maligna mais comumente diagnosticada em homens, atrás somente do câncer de pele não melanoma. Também é a segunda maior causadora de mortes, após somente dos tumores de pulmão. Fatores intrínsecos e extrínsecos contribuem para seu surgimento e progressão, como idade, raça, sedentarismo, hábitos alimentares e exposição a agentes biológicos e químicos, incluindo alcoolismo, tabagismo e manejo de pesticidas (INCA, 2020).

O uso dos praguicidas é uma das principais estratégias adotadas para garantir a produtividade de *commodities* agrícolas. Além disso, viabilizam o abastecimento de alimentos frente ao intenso crescimento da população mundial, sendo que esta pode aumentar em 2 bilhões de pessoas nos próximos 30 anos, de acordo com as Nações Unidas (UNITED NATIONS, 2020). O Brasil é um dos maiores produtores agropecuários do mundo, estando entre os países que mais exportam defensivos agrícolas e um dos líderes mundiais em consumo de pesticidas. Somente no ano de 2008, nosso país movimentou mais de US$ 7 bilhões na comercialização desses produtos. Em 2015, foram plantados 71,2 milhões de hectares de lavouras para 21 cultivares, sendo pulverizados 899 milhões

de litros de pesticidas. Os compostos mais utilizados entre os anos de 2012 e 2016 foram o inseticida Clorpirifós e os herbicidas Glifosato (GLI), com seu principal subproduto Ácido Aminometilfosfônico (AMPA), Atrazina (ATZ) e Ácido Diclorofenoxiacético **(**2,4-D) (PIGNATI et al., 2017).

Em junho de 2020, houve um salto nas taxas de exportação do agronegócio com uma elevação de 24,5% em relação ao mesmo mês do ano anterior, resultando em 10,17 bilhões de dólares em vendas externas, sendo 5,42 bilhões associados à soja (BRASIL, 2020). O crescimento também é acompanhado pelo uso de defensivos sendo que dados do Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - IBAMA (2019), revelaram que 122 empresas em 2018 comercializaram, internamente, 550 mil toneladas de ingredientes ativos de produtos químicos e bioquímicos, um incremento de 1,72% em relação a 2017. Os pesticidas que tiveram maior comercialização foram GLI e seus sais (sal potássico, sal de isopropilamina e sal de amônio), 2,4-D, Mancozebe e ATZ.

1.2 Hipótese

Diante da relevância epidemiológica do CaP e de seus fatores de risco, hipotetizamos que os herbicidas GLI e AMPA alterem a expressão de genes associados ao CaP, assim como modulem mecanismos relacionados à tumorigênese prostática.

1.3 Objetivos

1.3.1 Objetivo geral

Avaliar o comportamento celular e molecular de células prostáticas após tratamentos com os herbicidas GLI e AMPA.

1.3.2 Objetivos específicos

- Avaliar a citotoxicidade de GLI e AMPA em linhagens celulares prostáticos;
- Determinar a IC_{50} (concentração inibitória média) de cada herbicida frente as linhagens prostáticas malignas e não tumorigênicas;
- Determinar os mecanismos celulares pró-tumorais ativados nas células não-tumorigênicas (PNT2) após o tratamento com essas substâncias;

- Avaliar as alterações moleculares mediadas por estas moléculas na expressão dos genes Anexina A1 (*ANXA1*), Caderina 1 (*CDH1*), Gene 5 Específico para parada do crescimento (*GAS5*), Glutationa S-transferase Mu 3 (*GSTM3*), Interleucina 6 (*IL6*), Fator de crescimento transformador beta 1 (*TGFβ1*) e Vimentina (*VIM*) na linhagem PNT2;
- Compreender o comportamento destes químicos nas vias de promoção e progressão do CaP.

1.4 Justificativa

A estimativa de casos novos de câncer no Brasil é de 704 mil para cada ano do triênio 2023-2025, sendo que CaP representa 10,2 % dessa taxa. O CaP é um dos cânceres mais frequentes mundialmente, sendo o tumor maligno mais incidente em homens brasileiros, após o câncer de pele não melanoma (INCA, 2022). Embora os pesticidas apresentem um papel fundamental no aumento da produtividade e qualidade das culturas vegetais e na eliminação de vetores e pragas, podem afetar em recursos hídricos, agrícolas, animais e, inclusive, seres humanos (GALANI et al., 2021). A exposição a esses químicos pode acarretar a desregulação e perturbação da resposta imune, promovendo alterações na homeostase. Portanto, podem estar relacionados com o surgimento de diversas patologias, como o câncer (DONLEY et al., 2022; KAPELEKA et al., 2019; LEE; CHOI, 2020) . O efeito dos pesticidas GLI e AMPA sobre o CaP ainda não estão completamente elucidados. Portanto, torna-se imprescindível a compreensão das alterações moleculares mediadas por GLI e AMPA em células prostáticas bem como os mecanismos celulares pró-tumorais ativados nessas células após o tratamento com essas substâncias.

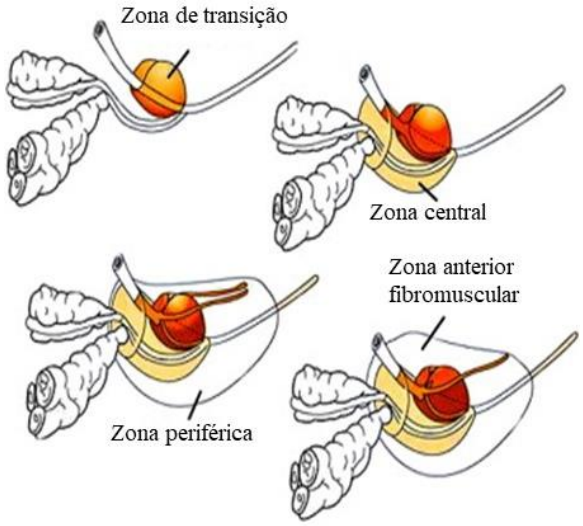
2 REFERENCIAL TEÓRICO

2.1 Câncer de Próstata: epidemiologia e aspectos gerais

A próstata é uma glândula sexual acessória no formato de uma noz que circunda o canal da uretra, situada em posição anterior ao reto, posterior à sínfise púbica e inferior a bexiga. A sua composição é aproximadamente 30% de elemento estromal e 70% glandular (LEE; AKIN-OLUGBADE; KIRSCHENBAUM, 2011). Sua função é a produção e secreção do líquido prostático que compõe de 20 a 30% do sêmen. Esse fluido alcalino, rico em minerais, citrato e enzimas, é responsável pela mobilidade e proteção dos espermatozoides, pois neutraliza o pH ácido da vagina, aumentando a fertilidade masculina (GÓMEZ et al., 2007; SARKAR; DAS, 2016; TOIVANEN; SHEN, 2017).

A glândula prostática é dividida em três regiões ou zonas de McNeal, quais sejam: a zona periférica (ZP), a zona de transição (ZT) e a zona central (ZC) **(**Figura 1**)**. A ZP constitui cerca de 70% do tecido glandular, a ZT em torno de 5% e a ZC cerca de 25%. Diferentemente das regiões glandulares (ZP, ZT e ZC), a zona anterior fibromuscular é constituída por tecido muscular fibroso (MCNEAL, 1981).

Figura 1: Representação das regiões da glândula prostática conhecidas como zonas de McNeal.



Fonte: Adaptado de (CRAWFORD, 2009).

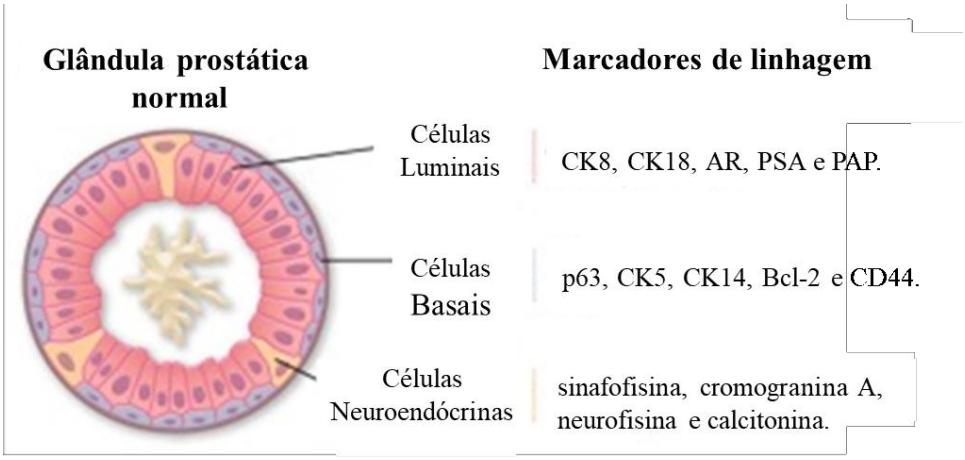
A incidência de prostatites, CaP e Hiperplasia Prostática Benigna (HPB) difere entre as regiões do órgão. Adenocarcinomas acometem, principalmente, a ZP,

contabilizando cerca de 70% dos casos, sendo 10% na ZC e 20% na ZT. Já a HPB é desenvolvida exclusivamente na ZT por se tratar de uma alteração no estroma fibromuscular (SARKAR; DAS, 2016).

Histologicamente, a próstata é constituída por um epitélio composto por células basais, neuroendócrinas e luminais (Figura 2). As basais apresentam forma arredondada e achatada, localizadas próximo à membrana basal dos ácinos. Expressam citoqueratinas (CK) de alto peso molecular, como a CK5 e CK14, e o marcador 63 (p63), utilizado no diagnóstico do CaP (SHEN; ABATE-SHEN, 2010; YOO et al., 2016). Expressam baixos níveis do receptor de andrógeno (AR), são andrógeno-independentes, mas responsivas a androgênio (TAYLOR; RISBRIDGER, 2008). Ademais, expressam a proteína Bcl-2 (*B-cell lymphoma protein 2*) e a glicoproteína transmembranar CD44 (LONG et al., 2005).

Em menor quantidade, as células neuroendócrinas estão espalhadas no tecido epitelial. Secretam peptídeos e hormônios como a sinafofisina, cromogranina A, neurofisina e calcitonina (VAN LEENDERS; SCHALKEN, 2003). Por outro lado, não expressam o AR e são andrógeno-independentes (TAYLOR; RISBRIDGER, 2008). Finalmente, as luminais são o tipo celular mais proeminente, com aspecto colunar e em contato com o lúmen. Contêm grânulos secretores de CKs de baixo peso molecular, como CK8 e CK18 (WANG et al., 2018), expressam altos níveis do AR, são andrógeno-responsivas e andrógeno-dependentes (TAYLOR; RISBRIDGER, 2008). Além disso, são responsáveis pela produção da enzima fosfatase ácida prostática (PAP) e do antígeno prostático específico (PSA), sendo que este solubiliza o ejaculado e, na prática clínica, é utilizado no rastreamento de tumores. Alterações na organização estrutural das células da próstata podem acarretar seu crescimento desordenado, o que promove a liberação do PSA na circulação sanguínea e o consequente aumento de seus níveis séricos (LILJA; ULMERT; VICKERS, 2008).

Figura 2: Representação do epitélio pseudoestratificado da próstata, sua constituição celular e seus respectivos marcadores. As células luminais secretam as citoqueratinas (CKs) 8 e 18 e expressam Receptor de Andrógeno (AR), Antígeno Prostático Específico (PSA) e Fosfatase Ácida Prostática (PAP). Já as basais expressam altos níveis da proteína 63 (p63), CK5, CK14, Bcl-2 e CD44.



Fonte: Adaptado de (WANG et al., 2018).

Quanto às patologias que acometem a próstata, estas podem ser benignas, como as prostatites e HPB, ou malignas. A prostatite é a inflamação da glândula prostática de homens adultos. As mais frequentes são de origem bacteriana aguda ou crônica, causadas, principalmente, por *Escherichia coli* e associadas a infecções urinárias. As menos frequentes são não bacterianas e prostatodinia, com causas ainda desconhecidas (SBU, 2020).

A HPB é um crescimento benigno do órgão ocasionado pelos componentes estromais e glandulares. Os sintomas são noctúria, alteração do jato urinário com a diminuição da força e calibre, sensação de urgência para urinar, micção frequente e incontinência. Assim como o CaP, a HPB acomete homens com idade mais avançada, geralmente acima dos 50 anos, com a taxa de prevalência superior a 20%, sendo que esta aumenta gradativamente com o passar dos anos (DING et al., 2013; IZUMI; LI; CHANG, 2014).

O CaP é o segundo câncer não cutâneo mais comumente identificado em homens, o segundo com maior mortalidade, após o câncer de pulmão. Em escala mundial é o quarto câncer com o maior número de óbitos (KAISER et al., 2019; MIRMOEENI et al., 2022). Segundo as projeções do Instituto Nacional de Câncer (2022), o número estimado de novos casos de CaP para o Brasil, para cada ano do triênio 2023-2025, é de aproximadamente 72 mil, representando 30 % de todas as neoplasias no sexo masculino. As taxas elevadas se devem à melhoria das técnicas de diagnóstico, aos investimentos em

políticas públicas de divulgação e ao aumento da qualidade de vida e, consequentemente, da expectativa de vida. Para o ano de 2022, nos Estados Unidos, são esperados cerca de 268 mil novos casos e 35 mil mortes por essa doença (AMERICAN CANCER SOCIETY, 2022).

O rastreamento regular, a partir dos 50 anos de idade, é de suma importância para o diagnóstico precoce do CaP, visto que o crescimento do tumor é lento e, em fase inicial, geralmente assintomático. A detecção precoce é fundamental para maior chance de cura e para a eficácia terapêutica. Portanto, pesquisas voltadas para o desenvolvimento de novos métodos de diagnóstico e para a caracterização de alterações moleculares-chave se destacam (MINISTÉRIO DA SAÚDE, 2020).

Rotineiramente, é realizado o toque retal (TR), um exame digital indicado para homens com idade a partir de 50 anos e, para pacientes com histórico familiar, a partir 45 anos. O TR é simples, de baixo custo e com pequenas taxas de complicações (ANDRIOLE et al., 2009; NASCIMENTO; FLORINDO; CHUBACI, 2011). São avaliadas a dimensão, forma e consistência do órgão. Contudo, somente as porções lateral e posterior da glândula podem ser palpadas, não detectando de 40 a 50% dos tumores (JANUÁRIO et al., 2015). A fim de aumentar a acurácia dos exames diagnósticos recomenda-se associar o TR com a dosagem do PSA, o que culmina com o aumento da sensibilidade para 95% (BRASIL, 2002).

No século XX, o trabalho de Wang e colaboradores (1979) foi pioneiro em purificar e caracterizar o PSA por meio da técnica de imunoprecipitação, definindo-o, na época, como próstata-específico. Pouco tempo depois, em 1986, o teste de dosagem dos níveis séricos de PSA foi aprovado pela *Food and Drug Administration* (FDA) para acompanhar a progressão dos tumores em pacientes já diagnosticados com CaP e para monitorar a resposta e eficácia terapêuticas. Posteriormente, a FDA aprovou sua associação com o TR para o diagnóstico da doença (KOHAAR; PETROVICS; SRIVASTAVA, 2019).

O PSA ou calicreína humana 3 (hK3) é uma enzima proteolítica com 33 kDa produzida e secretada pelo epitélio prostático e codificada pelo gene *KLK3* localizado no cromossomo 19q 13.3–13.4. É um biomarcador incorporado à rotina clínica, contudo com baixa especificidade para a detecção de tumores, uma vez que seus níveis também estão elevados em outras condições fisiopatológicas como prostatite e HPB, ou em situações esporádicas como após ejaculação e cateterismo. Ademais, o PSA não consegue

distinguir as formas indolente da agressiva dos tumores (FILELLA; FOJ, 2016; HATAKEYAMA et al., 2017).

Ao serem detectadas anormalidades no TR e nos níveis de PSA sérico, com suspeita de malignidade, a biópsia é realizada para a confirmação do CaP. Majoritariamente, a técnica é guiada por ultrassonografia transretal. Após confirmação, as peças são analisadas histopatologicamente e estadiadas para que, então, seja definido o melhor método terapêutico (BORGHESI et al., 2017; SALAMI et al., 2015).

Os tumores prostáticos são estadiados conforme o sistema *Tumor Node Metastasis* (TNM) e classificados de acordo com o Escore de Gleason (EG), o que define a agressividade tumoral e o prognóstico do paciente (SEPÚLVEDA et al., 2014). O TNM avalia e caracteriza a extensão do tumor primário (T), comprometimento de linfonodos próximos (N) e a presença de metástases (M). Os níveis de PSA sérico e EG também são considerados (ONCOGUIA, 2020). Este baseia-se na arquitetura celular do tumor e estabelece cinco padrões histológicos dissemelhantes, variando do grau 1 bem diferenciado ao 5 indiferenciado. O EG é dado pelo somatório dos dois padrões predominantes na peça resultando em pontuação variando de 2 a 10 (SEPÚLVEDA et al., 2014). Em 2014, o EG foi atualizado pela Sociedade Internacional de Patologia Urológica com a definição de cincos categorias:: (1) escore ≤ 6 (3+3) com organização glandular individual, bem formada e discreta; (2) escore 7 (3+4) com glândulas predominantemente bem formadas com poucas malformadas, fundidas e cribriformes; (3) score 7 (4+3) com aspecto glandular majoritariamente malformado, fundido e cribriforme; (4) score 8 com glândulas bem formadas (3+5) ou mal formadas, fundidas e cribiformes (4+4) ou glândulas predominantemente ausentes (5+3); e (5) score 9 ou 10 em que não há formação glandular ou estas encontram-se necrosadas (EPSTEIN, 2018).

A escolha da opção terapêutica para cada paciente depende, portanto, do estágio do tumor de acordo com a classificação TNM e o EG, da presença de comorbidades e dos níveis de PSA (PARTIN et al., 1997). Dentre os métodos disponíveis destacam-se prostatectomia radical (PR), radioterapia, hormonioterapia e quimioterapia (AMERICAN CANCER SOCIETY, 2019; WILT et al., 2008).

A PR é o método indicado em casos de CaP localizado de baixo risco e intermediário, com expectativa de vida superior a 10 anos. Trata-se da remoção cirúrgica parcial ou total da próstata e vesículas seminais (HEIDENREICH et al., 2014). A fim de eliminar o tumor localizado, ou retardar seu crescimento, administra-se a radioterapia externa por meio de radiações ionizantes, como o raio X de alta intensidade, ou a

radioterapia interna (braquiterapia) através da implantação de sementes radioativas de baixa ou alta taxa de dose (DEARNALEY et al., 2016).

A hormonioterapia objetiva conter ou bloquear a progressão dos tumores por meio da ablação dos níveis dos hormônios androgênicos, responsáveis pela indução do crescimento celular tumoral prostático. Os principais andrógenos são testosterona e diidrotestosterona (DHT) e a terapia de privação androgênica (ADT) pode ocorrer por castração cirúrgica ou química (ou medicamentosa). Na primeira há a remoção dos testículos, procedimento este denominado orquiectomia bilateral. Na segunda, podem ser administrados medicamentos que bloqueiam a ação dos hormônios gonadotróficos FSH (hormônio folículo estimulante) e LH (hormônio luteinizante), atuando na produção da testosterona. Também são utilizados agonistas do receptor do hormônio liberador do hormônio luteinizante (LHRH), antagonistas do AR, inibidores de CYP-17 e estrogênio. A ADT está associada a diversos efeitos colaterais físicos e psicológicos, como ginecomastia, fadiga, disfunção sexual, anemia, fraqueza muscular, distúrbios emocionais e dificuldades cognitivas (CHEUNG et al., 2017; CHUNG et al., 2017). Apesar de eficaz, alguns pacientes adquirem resistência aos medicamentos utilizados, progredindo para o CaP resistente à castração (CPRC) (NADER; EL A MM; ARAGON-CHING, 2018). Nesses casos, são utilizados quimioterápicos por via intravenosa ou oral, como cabazitaxel, docetaxel, mitoxantrona e estramustina (EVANS, 2018).

Quimioterápicos, por sua vez, apresentam atuação sistêmica, sendo citotóxicos a células de multiplicação acelerada. Porém, carecem de especificidade afetando o revestimento bucal e os folículos pilosos, causando efeitos colaterais como alopecia e inflamações bucais, assim como falta de apetite, náuseas, vômitos, fadiga, entre outros (ONCOGUIA, 2020).

2.2 A etiopatologia de tumores prostáticos

O CaP é uma doença complexa, heterogênea e multifatorial. O processo de carcinogênese inclui, de forma geral, três etapas: (i) Iniciação, quando as células, sob ação de um carcinógeno ou agente oncoiniciador, sofrem alterações em genes-chave como supressores tumorais e proto-oncogenes. Nesse estágio as células já estão modificadas geneticamente, no entanto não é possível detectar clinicamente a formação do tumor; (ii) Promoção, na qual as células já modificadas geneticamente sofrem os efeitos de oncopromotores e (iii) Progressão, com a proliferação desordenada das células

transformadas. Nessa etapa, a lesão evolui até a manifestação dos primeiros sintomas da doença (ANAND et al., 2008; INCA, 2019). Diferentes fatores de risco contribuem para o processo de carcinogênese, como idade, predisposição genética, histórico familiar e etnia. Adicionalmente, hábitos alimentares e exposição a carcinógenos também estão associados ao seu desenvolvimento (PERNAR et al., 2018).

A idade é o fator de risco mais bem estabelecido. Homens acima de 65 anos correspondem a dois terços dos diagnósticos dessa doença (PERDANA; MOCHTAR; UMBAS, 2016). A partir de 67 anos, a chance de desenvolver CaP aumenta em cerca de 35% (GROZESCU; POPA, 2017). De fato, mutações no DNA são responsáveis pelo surgimento desses tumores, as quais são mais frequentes com o decorrente comprometimento da função molecular associado ao avanço da idade. As somáticas são as mais comuns e ocorrem em 80 a 90% dos casos. Quando as alterações são germinativas, podem ser transmitidas hereditariamente, contabilizando de 10 a 20% dos casos. Nesse contexto, as lesões são identificadas em homens mais jovens, de modo que 43% dos diagnósticos ocorrem naqueles com menos de 70 anos e apenas 9% em pacientes com idade superior aos 85 anos. De fato, quando hereditário, o CaP tem manifestação precoce, sendo detectado de 6 a 7 anos mais cedo (FERRÍS-I-TORTAJADA et al., 2011). Quanto às mutações, destacam-se as identificadas nos genes supressores tumorais *BRCA1* (*Breast Cancer 1*) *e BRCA 2* (*Breast Cancer 2*), também relacionadas aos cânceres de mama (CM) e ovário (NYBERG et al., 2020). Finalmente, o CaP também está associado ao número de parentes de primeiro e segundo graus já diagnosticados com a doença. Quando há um familiar de primeiro grau com CaP, o risco relativo é 2,48 para o desenvolvimento da lesão; se houver dois ou mais familiares doentes, o risco relativo aumenta para 4,39, quando comparados homens de mesma idade, localização geográfica e etnia (MERRIEL; FUNSTON; HAMILTON, 2018).

A etnia também é relacionada ao CaP, com incidência e mortes elevadas entre negros. Estudos mostram que polimorfismos de nucleotídeo único (SNPs) em alelos de risco no cromossomo 8q24 estão associadas ao risco de CaP e o SNP Broad11934905 é encontrado somente em pessoas com ascendência africana (LAYNE et al., 2019; POWELL; BOLLIG-FISCHER, 2013; WHITMAN et al., 2010). Alguns pesquisadores demostraram diversas variações nos genes de apoptose celular, como *BCL-2*, e em supressores tumorais, como *EPHB2* (Receptor 2 da Efrina tipo B), em homens negros (PERDANA; MOCHTAR; UMBAS, 2016), o que pode explicar sua propensão à doença.

Considerando os fatores extrínsecos, os hábitos alimentares se destacam. De fato, o desenvolvimento de CaP é influenciado por alterações metabólicas advindas da alimentação. O consumo de gordura saturada, carne vermelha, cálcio e a obesidade estão relacionadas à rápida progressão, aumento das taxas de mortalidade, fenótipo mais agressivo e elevada taxa de recorrência (LABBÉ et al., 2019). Já a exposição a agentes biológicos, físicos e químicos também deve ser considerada e sistematicamente estudada.

Os vírus oncogênicos são responsáveis de 10 a 15% dos diagnósticos de cânceres em humanos. Esses patógenos utilizam a maquinaria da célula hospedeira para a produção de novas partículas virais. As células infectadas, portanto, passam a expressar genes virais que atuam na indução da proliferação e crescimento celulares, inibindo a apoptose. A interação dos vírus com o genoma do hospedeiro pode acarretar inflamação crônica ou recorrente da próstata, ou transformar as células hospedeiras em tumorais. Papilomavírus humano (HPV), poliomavírus como vírus BK (BK), vírus John Cunningham (JC) e vírus vacuolante símio 40 (SV40) e alguns herpes-vírus como Citomegalovírus Humano (HCMV) e vírus Epstein-Barr (EBV) estão relacionados a infecções prostáticas e ao CaP (CHEN; WEI, 2015; LAWSON; GLENN, 2020). Adicionalmente, bactérias também são associadas à doença. A bactéria gram-positiva *Propionibacterium acnes* tem sido detectada significativamente em maior concentração em pacientes com CaP quando comparados a indivíduos saudáveis, sugerindo sua relação com carcinogênese prostática. A infecção por *P. acnes* induz a uma intensa secreção de citocinas inflamatórias, as interleucinas 6 (IL6) e IL8, comprovadamente pró-tumorais (DAVIDSSON et al., 2016).

Carcinógenos físicos como radiação ionizantes e não ionizantes (raios ultravioletas, raios X e raios gama) são denominados agentes de iniciação, pois danificam o material genético induzindo à transformação maligna (COOPER, 2000). Já os carcinógenos químicos são moléculas eletrofílicas que modificam o material genético por meio de interações do tipo covalente, as quais induzem erros durante a fase de replicação, favorecendo a formação de células malignas. Estes compostos podem ser categorizados em diretos, quando reagem diretamente com DNA, ou indiretos (pré-carcinógenos), quando necessitam ser metabolizados para se tornarem um agente causador de dano efetivo (BARROS; PAVÃO, 2010). Baseando em estudos epidemiológicos e experimentais a *International Agency for Research on Cancer* (IARC) classifica os compostos químicos de acordo com o potencial carcinogênico em quatro grupos demonstrados na Tabela 1 (INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, 2006):

Tabela 1: De acordo com a *International Agency for Research on Cancer* (IARC) os compostos químicos podem ser classificados em: (I) Carcinogênicos em humanos, (IIA) Prováveis carcinogênicos em humanos, (IIB) Possíveis carcinogênicos em humanos, (III) Não podem ser classificados como carcinogênicos e (IV) Não carcinogênicos em humanos.

Grupo	Classificação	Evidências
I	Carcinogênicos em humanos	Provas experimentais de carcinogenicidade suficientes em humanos e animais de laboratório
IIA	Prováveis carcinogênicos em humanos	Evidências limitadas de carcinogenicidade em humanos e satisfatórias em animais de laboratório
IIB	Possíveis carcinogênicos em humanos	Evidências limitadas de carcinogenicidade em humanos e não satisfatórias em animais de laboratório
III	Não podem ser classificados como carcinogênicos	Evidências inadequadas de carcinogenicidade em humanos e animais de laboratório
IV	Não carcinogênicos em humanos	Evidências que sugerem falta de carcinogenicidade em humanos e animais de laboratório

Fonte: (INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, 2006).

Nesse contexto, destacam-se as substâncias presentes no fumo (nitrosaminas e cádmio), as quais aumentam os níveis plasmáticos de testosterona total e livre, além de ativarem mecanismos epigenéticos relacionados a um perfil mais agressivo do CaP e induzirem a angiogênese e a proliferação das células malignas (KENFIELD et al., 2011). Portanto, o tabagismo aumenta o risco do surgimento do câncer e de um fenótipo mais agressivo da doença com fatalidade superior a 30%. Pesticidas também têm sido relacionados a tumores com potencial mutagênico e imunossupressor. Essas substâncias persistem no ambiente e podem contaminar recursos hídricos e alimentícios. A intoxicação por esses químicos pode acontecer por via oral (ingestão dos alimentos), via respiratória (inalação de fumaças) ou por via dérmica (contato direto com a pele). Por esta razão, pesticidas organoclorados são proibidos em países como China, Portugal e Suécia (BELPOMME et al., 2007; CRUZ; LINO; SILVEIRA, 2003; SANTOS et al., 2006; WONG; LEE, 1997).

2.3 Pesticidas e seu papel controverso na oncologia

A Lei Federal nº 7.802/89, regulamentada pelo Decreto 4.074 de 4 de janeiro de 2002, define os pesticidas como:

> Produtos e agentes de processos físicos, químicos ou biológicos, destinados ao uso nos setores de produção, no armazenamento e beneficiamento de produtos agrícolas, nas pastagens, na proteção de florestas, nativas ou implantadas, e de outros ecossistemas e de ambientes urbanos, hídricos e industriais, cuja finalidade seja alterar a composição da flora ou da fauna, a fim de preservá-las da ação danosa de seres vivos considerados nocivos, bem como as substâncias e produtos empregados como desfolhantes, dessecantes, estimuladores e inibidores de crescimento (PERES; MOREIRA, 2003, p. 24).

Os praguicidas são regulamentados no Brasil desde a década de 30 com a promulgação do Decreto nº 24.114 no ano de 1934 que vigorou as orientações e obrigações relacionadas à produção, importação, exportação, comercialização e utilização desses compostos no território brasileiro (REBELO; CALDAS, 2014). Essas substâncias são classificadas de acordo com a aplicação, origem e estrutura química. Quanto ao modo de ação no organismo alvo podem ser inseticidas, rodenticidas, herbicidas, fungicidas, fumigantes, nematicidas, ovicidas, larvicidas e acaricidas (WISMER; MEANS, 2018). Os defensivos podem ser orgânicos ou inorgânicos segundo sua composição de origem. Os orgânicos são subdivididos entre os de origem vegetal (piretrina, sabatina, rotenona e nicotina) e os organossintéticos que, por sua vez, se subdividem em organoclorados, fosforados, carbamatos, triazinas, piretroides e cloroacetamidas. Os inorgânicos são compostos de metais tóxicos como chumbo, mercúrio, cádmio e arsênio (SAVOY, 2011).

Os organoclorados apresentam, no mínimo, um átomo de cloro ligado covalentemente à cadeia carbônica. São hidrofóbicos e altamente lipossolúveis, podendo, portanto, se acumular em tecidos gordurosos dos organismos vivos. Dentre os mais consumidos destacam-se o Dicloro-Difenil-Tricloroetano (DDT), aldrina e 2,4-D. Em consequência da persistência ambiental e impacto em diferentes espécies animais, o DDT foi proibido na década de 70 na maior parte dos países desenvolvidos. No Brasil, o DDT e outros organoclorados foram impedidos de serem comercializados na década de 80 (DELGADO et al., 2002). Os organofosforados são derivados do ácido fosfórico, tiofosfórico ou ditiofosfórico e empregados no combate a ácaros, nematoides e fungos. Os que mais se destacam são Diclorvós, Clorpirifós e GLI. Os carbamatos derivam de compostos nitrogenados, do ácido carbâmico como o Carbaril e Propoxur. Já as triazinas

são empregadas como herbicidas inibindo a fotossíntese durante as fases pré e pós-emergente. Pode-se destacar a ATZ, a cianazina e simazina (GARBELLINI et al., 2007). Finalmente, os piretroides são derivados sintéticos das piretrinas, encontradas nas flores de *Chrysanthemum cinerariefolium* e *Chrysanthemum cocineum*. São usualmente manipulados como inseticidas e possuem baixa toxicidade em mamíferos, destacando-se cipermetrina e deltametrina (TRAMUJAS et al., 2006).

Os praguicidas apresentam alto grau de toxicidade e, quando usados exacerbadamente e/ou de maneira frequente, geram danos ambientais afetando solo, ar e água, além de causarem prejuízos à saúde humana e animal (JOBIM et al., 2010). Compreender esses efeitos adversos sobre os organismos e estabelecer níveis seguros de utilização torna-se imperativo. Portanto, é realizada a categorização toxicológica em relação aos efeitos na saúde humana com base na dose necessária para matar 50% da população teste (DL50), por via oral. Os valores correspondem à quantidade, em miligramas, do ingrediente ativo do pesticida por quilograma do peso do animal teste (EMBRAPA, 2020) (Tabela 2). Quanto à periculosidade ambiental (PPA) são considerados os parâmetros de potencial mutagênico, teratogênico e carcinogênico, bioacumulação, transporte, persistência e toxicidade a diversos organismos, sendo categorizados em I: produtos altamente perigosos; II: produtos muito perigosos; III: produtos perigosos e IV: produtos pouco perigosos (PERES; MOREIRA, 2003).

Tabela 2: Classificação toxicológica dos agrotóxicos frente aos riscos à saúde humana.

Classe	DL50 (mg/kg)	Cor da faixa
I- Produto Extremamente Tóxico	≤ 5	Vermelha
II- Produto Altamente Tóxico	5-50	Amarela
III- Produto Moderadamente Tóxico	50-500	Azul
IV- Produto Pouco Tóxico	50-5000	Verde

Fonte: EMBRAPA, 2020.

O alto índice de óbitos por autointoxicação por ingestão de pesticidas é preocupante, sendo que são contabilizados em torno de 260 a 370 mil por ano em todo o mundo. Majoritariamente, os casos são mais recorrentes em áreas rurais carentes com poucas informações disponíveis sobre a manipulação correta destes compostos (MOEBUS; BOEDEKER, 2017). Devido à falta de instruções e a não seletividade de diversos pesticidas, trabalhadores e profissionais da saúde pública são expostos aos riscos inerentes, como mutações no DNA e desenvolvimento de neoplasias. O restante da

população pode ser afetado, em menores proporções, por via oral ao ingerir alimentos e água contaminados (WHO, 2020).

Os pesticidas estão associados ao desenvolvimento de diversas doenças por promoverem alterações epigenéticas, genotoxicidade, alteração no reparo do DNA, estresse oxidativo (EO), imunossupressão e mudanças nos mecanismos de controle da proliferação celular e morte celulares (COSTA; MELLO; FRIEDRICH, 2017). Especial atenção tem sido dedicada a tumores como de próstata e de mama, além de também serem relatados distúrbios no sistema nervoso central (SNC) e periférico (SNP), doenças respiratórias e transtorno do espectro do autismo (TEA) (CLAPP; JACOBS; LOECHLER, 2008; VALCIN et al., 2007).

O contato com praguicidas influencia a atividade enzimática da glutationa-S-transferase (GST), interferindo na capacidade antioxidante das células e conduzindo à neurotoxicicidade. De fato, o EO ocasiona injurias celulares e propicia o desenvolvimento de doenças neurodegenerativas, como a doença de Parkison (DP). Quando a exposição a esses químicos é duradoura o risco de DP é aumentado em 11% (ULLAH et al., 2021). Adicionalmente, exposições pré-natais e infantis, especialmente no primeiro ano de vida da criança, a pesticidas neurotóxicos podem aumentar o risco do desenvolvimento de TEA. Em estudos experimentais utilizando camundongos com traços comportamentais de TEA expostos ao inseticida Clorpirifós, foi verificada um aumento no EO cerebral e na síntese de prostaglandina E2. Pesticidas piretroides e organoclorados, como cipermetrina e endosulfan, são altamente tóxicos e causam anomalias neurocomportamentais por modificarem a expressão de neuroproteínas (LEE et al., 2015; VON EHRENSTEIN et al., 2019).

O manuseio demasiado, incorreto e sem a utilização de Equipamentos de Proteção Individual (EPIs) faz com que a classe de trabalhadores rurais tenha maior chance de desenvolver doenças, principalmente as respiratórias como bronquite crônica, asma e respiração ofegante. Durante a pulverização dos produtos químicos, são liberados aerossóis que, se inalados e/ou absorvidos, podem atingir os tecidos pulmonares causando irritabilidade e a constrição das vias aéreas (PRIYADHARSHINI et al., 2017). Considerando os possíveis prejuízos causados pelo manejo de pesticidas, torna-se também necessária a avaliação sistemática de seus efeitos e sua relação com o desenvolvimento de tumores. No caso de trabalhadores em lavouras, estes estão em contato com diversos agentes físicos, químicos e biológicos, majoritariamente, pesticidas, radiação ionizante, solventes, poeiras minerais e orgânicas e microrganismos, o que

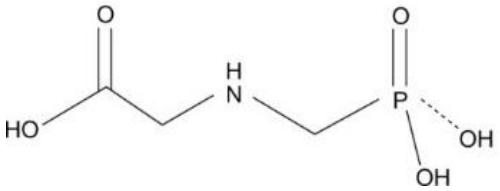
desafia a identificação dos seus efeitos individuais. Contudo, tem sido demonstrada a relação dos pesticidas com o CaP (VAN MAELE-FABRY; WILLEMS, 2004). Segundo a literatura, o risco de carcinogênese prostática agressiva pode ser aumentado quando há o contato com organofosforados, fonofos, malathion, terbufos e o organoclorado aldrin, principalmente em indivíduos com histórico familiar da doença. Esses compostos químicos atuam alterando vias de sinalização envolvidas na adesão, proliferação e diferenciação celulares, além de serem genotóxicos e desreguladores endócrinos, podendo acumular nas células adiposas e gerar uma perturbação metabólica contínua (KOUTROS et al., 2013). Pesquisas demonstram que alguns organoclorados como diclorodifenildicloroetano (DDE) e DDT podem alterar as vias de progressão do CM e consequentemente, o prognóstico e a taxa de sobrevida. O DDT altera o equilíbrio hormonal estrógeno- andrógeno, induzindo a proliferação de células tumorais. O risco de morte entre as pacientes ER-positivas é elevado quando são expostas ao organoclorado dieldrin (KOUAL et al., 2020).

Nesse cenário, compreender as alterações mediadas por esses produtos se mostra, portanto, essencial, para que, assim, seu uso seja racional e seguro (MOEBUS; BOEDEKER, 2017). Além disso, o conhecimento decorrente desse estudo poderá subsidiar campanhas de conscientização e otimizar ações voltadas para o controle de sua utilização, principalmente dos mais utilizados e comercializados no Brasil como o GLI, AMPA, ATZ e o 2,4-D.

2.3.1 Glifosato e Ácido Aminometilfosfônico

O GLI [*N* - (fosfonometil)glicina)] é um composto organossintético pertencente ao grupo químico organofosforado. Sua síntese ocorre a partir do aminoácido glicina, com a alteração de um hidrogênio amínico por um radical metilfosfônico. Sua fórmula molecular é $C_3H_8NO_5P$ (Figura 3) com massa de 169,07 g mol $^{-1}$. Em condições normais é sólido cristalino, quimicamente estável. Sua solubilidade em água é 12 g L^{-1} a 25 ° C, o ponto de fusão é a 200 ° C, a densidade aparente de 0,5 g/cm^3. Possui elevada estabilidade à luz e com meia vida variando entre 5 a 23 dias em solos (AMARANTE JUNIOR et al., 2002; DOMÍNGUEZ et al., 2016; LI et al., 2013).

Figura 3: Estrutura química do composto glifosato.



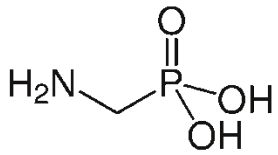
Fonte: (LI et al., 2013).

É um pesticida sistêmico, pós-emergente e não seletivo, utilizado como herbicida e dessecante de amplo espectro (GOMES et al., 2014). Foi lançado em 1974 e seu uso aumentou drasticamente após o desenvolvimento de plantas geneticamente modificadas resistentes a esse produto. Na atualidade, é o princípio ativo mais produzido e pulverizado mundialmente, visando erradicar pragas e controlar ervas daninhas. O GLI é aplicado em áreas agrícolas, urbanas e residenciais (ANDREOTTI et al., 2018; DE ARAUJO; DELGADO; PAUMGARTTEN, 2016).

O GLI é classificado como provável carcinógeno para humanos (Grupo 2A) e estudos apresentaram evidência limitada quanto ao risco para o desenvolvimento de Linfoma não-Hodgkin em humanos, apesar de causar a morte de camundongos. Esse químico foi responsável por ocasionar danos ao DNA e alterações cromossômicas em células humanas, além de promover um aumento no número de micronúcleos em moradores de regiões onde o herbicida foi pulverizado (IARC, 2015).

A degradação biológica do GLI é realizada por microrganismos presentes no solo e organismos saprófitos por meio de duas rotas metabólicas. Uma envolve a clivagem oxidativa da ligação carbono-nitrogênio (C-N) para produzir seu principal metabólito, o ácido aminometilfosfônico (AMPA) (MAMY; BARRIUSO; GABRIELLE, 2016). O AMPA é um ácido orgânico fraco comercializado em pó com fórmula molecular CH_6NO_3P (Figura 4**)**, massa de 111,04 g mol^{-1}, baixa toxicidade, com DL50 de 8.300 mg kg^{-1}, temperatura de fusão 120 º C e solubilidade em água 50 g L^{-1} (SOUZA et al., 2006). Em relação ao GLI, o AMPA é considerado mais persistente em solos por possuir meia vida superior, oscilando entre 76 a 240 dias, o que pode aumentar os riscos de toxicidade (ANNETT; HABIBI; HONTELA, 2014; DOMÍNGUEZ et al., 2016).

Figura 4: Estrutura química do Ácido Aminometilfosfônico (AMPA).



Fonte: (ANNETT; HABIBI; HONTELA, 2014)

A segunda rota de degradação de GLI envolve a enzima C-P liase com a ruptura da ligação fósforo-carbono (C-P) gerando um metabólito intermediário, a sarcosina (REDDY; RIMANDO; DUKE, 2004). O GLI impede o crescimento das plantas indesejáveis interferindo na síntese proteica ao inibir a atividade da enzima 5-enolpiruvilshiquimato-3-fosfato sintase (EPSPS), essencial para a via do chiquimato. A EPSPS catalisa a biossíntese dos aminoácidos aromáticos fenilalanina, tirosina e triptofano em plantas, algas, fungos e bactérias (FARIA et al., 2019).

CAPÍTULO 2

GLYPHOSATE AND AMINOMETHYPHOSPHONIC (AMPA) MODULATE GLUTATHIONE S-TRANSFERASE IN PROSTATE CELLS

Dayanne Silva Borges, Deysse Carla Tolentino Barros, Vinícius Marques Arruda, Matheus Fernandes da Silva, Joyce Ferreira da Costa Guerra, Raoni Pais Siqueira, Thaise Gonçalves Araújo

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Glyphosate and Aminomethylphosphonic Acid (AMPA) Modulate Glutathione S-Transferase in Non-Tumorigenic Prostate Cells

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Abstract: Glyphosate (GLY) was developed in the early 1970s and has become the most used broad-spectrum herbicide in the world so far. Its main metabolite is aminomethylphosphonic acid (AMPA), and the accumulation of GLY and its derivative compounds raises some concerns regarding possible health outcomes. In this study, we aimed to evaluate the effects of GLY and AMPA on prostate cell lines by evaluating cell viability, proliferation, gene and protein expression, and cellular pathways involved in the response to oxidative stress. Our results indicated that GLY and AMPA reduced the cell viability of tumorigenic and non-tumorigenic prostate cell lines only at higher concentrations (10 mM GLY and 20 mM AMPA). In contrast, both compounds increased the clonogenicity of non-tumorigenic PNT2 cells, mainly at concentrations below the IC_{50} (5 mM GLY and 10 mM AMPA). Moreover, treatment of non-tumorigenic cells with low concentrations of GLY or AMPA for 48 h increased GSTM3 expression at both mRNA and protein levels. In contrast, the treatments decrease the GST activity and induced an increase in oxidative stress, mainly at lower concentrations. Therefore, both compounds can cause cellular damage even at lower concentrations in non-tumorigenic PNT2 cells, mainly affecting cell proliferation and oxidative stress.

Keywords: prostate; organophosphate; glyphosate; aminomethylphosphonic acid; metabolism; transcripts

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1. Introduction

Brazil is the fifth largest agricultural producer worldwide, being the main producer of coffee, sugarcane, and citrus [1]. Since agriculture is the leading contributor to the Brazilian economy, special attention must be paid to the use of synthetic chemicals and their economic, ecological, and health consequences [2]. While pesticides and herbicides have helped Brazil to become one of the top-producing countries, their use has raised several questions about their real safety. With an estimated world population of 8.5 billion in 2030, the demand for food is notorious, which highlights the need to fight weeds to increase agricultural production [3]. However, the excessive use of pesticides and herbicides results in the contamination of the soil and water, affecting the whole ecosystem [4].

Glyphosate (N-(phosphonomethyl)glycine-GLY) is a non-selective herbicide with broad-spectrum activity. It has been used worldwide in agriculture, forestry, and industry for weed control [5,6]. This compound interferes with the shikimate pathway in plants and microorganisms, thereby inhibiting the synthesis of aromatic amino acids [7]. GLY-based

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CONCLUSÃO

GLI e AMPA são ativos em linhagens prostáticas, se mostrando mais citotóxicos para a linhagem CaP hormônio dependente. Os compostos só foram ativos à linhagem PC-3 nas maiores concentrações. Na linhagem PNT2, o IC50 foi próximo ao das células LNCaP, contudo, quando GLI e AMPA foram utilizados nas concentrações inferiores aos valores do IC50 nas células não tumorigênicas induziram a proliferação celular, superexpressão do gene que codifica para GSTM3, assim como seus níveis proteicos. No entanto, a atividade enzimática de GST foi significativamente reduzida, com a elevação significativa da oxidação das proteínas. A modulação de *GAS5* só foi detectada nos tratamentos com 10 mM GLI e 20 mM AMPA, os quais podem estar relacionados aos mecanismos de morte estimulados pelas concentrações mais altas. Portanto, não se mostraram evidentes os efeitos dos químicos na modulação dos transcritos de *GSTM3*, mas sim, em sua atividade enzimática.

Demostramos que, mesmo em concentrações não citotóxicas, há efeitos dos pesticidas no metabolismo celular que podem estar envolvidos com o processo da carcinogênese prostática. Portanto, é indispensável estudos adicionais para a melhor compreensão dos efeitos e na modulação das vias com tratamentos em concentrações residuais, visando compreender os efeitos biológicos dos pesticidas.

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ANEXO 1

Normas para publicação de artigo no periódico *International Journal of Molecular Sciences* (IJMS)

Instructions for Authors

Shortcuts

- **Manuscript Submission Overview**
- **Manuscript Preparation**
- **Preparing Figures, Schemes and Tables**
- **Original Images for Blots and Gels Requirements**
- **Supplementary Materials, Data Deposit and Software Source Code**
- **Research and Publication Ethics**
- **Reviewer Suggestions**
- **English Corrections**
- **Preprints and Conference Papers**
- **Authorship**
- **Editorial Independence**
- **Conflicts of Interest**
- **Editorial Procedures and Peer-Review**
- **Promoting Equity, Diversity and Inclusiveness Within MDPI Journals**
- **Resource Identification Initiative**

Submission Checklist

Please:

1. Read the **Aims & Scope** to gain an overview and assess if your manuscript is suitable for this journal;
2. Use the **Microsoft Word template** or **LaTeX template** to prepare your manuscript;
3. Make sure that issues about **publication ethics**, **research ethics**, **copyright**, **authorship**, **figure formats**, **data** and **references format** have been appropriately considered;
4. Ensure that all authors have approved the content of the submitted manuscript.
5. Authors are encouraged to add a **biography** (optional) to the submission and post it to **SciProfiles**.

Manuscript Submission Overview

Types of Publications

IJMS has no restrictions on the length of manuscripts, provided that the text is concise and comprehensive. Full experimental details must be provided so that the results can be reproduced. *IJMS* requires that authors publish all experimental controls and make full datasets available where possible (see the guidelines on **Supplementary Materials** and references to unpublished data).

Manuscripts submitted to *IJMS* should neither be published previously nor be under consideration for publication in another journal. The main article types are as follows:

- *Articles:* Original research manuscripts. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information. Authors should not unnecessarily divide their work into several related manuscripts, although short *Communications* of preliminary, but significant, results will be considered. The quality and impact of the study will be considered during peer review.
- *Reviews:* The journal encourages the authors to submit comprehensive reviews on specific topics. Reviews should provide a complete and balanced overview of the latest progress in a given area of research. Reviews should not (1) merely summarize the literature or (2) focus on authors' own work or that of their close colleagues. Unpublished research data are not allowed. Systematic reviews should follow the PRISMA **guidelines.** More details can be found at **LINK**.

Submission Process

Manuscripts for *IJMS* should be submitted online at **susy.mdpi.com**. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the **criteria to qualify for authorship**) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the **submission website**. Once you have registered, **click here to go to the submission form for *IJMS***. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

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- *Microsoft Word:* Manuscripts prepared in Microsoft Word must be converted into a single file before submission. When preparing manuscripts in Microsoft Word, we encourage you to use the ***IJMS* Microsoft Word template file**. You must insert your graphics (schemes, figures, etc.) in the main text after the paragraph in which it is first cited. You may, in addition, upload them during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.
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- When your manuscript reaches the revision stage, you will be requested to format the manuscript according to the journal guidelines.

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A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work. It should explain why the manuscript fits the scope of the journal.

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- We confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal.
- All authors have approved the manuscript and agree with its submission to (journal name).

Author Biography

Authors are encouraged to add a biography (maximum 150 words) to the submission and post it to **SciProfiles**. This should be a single paragraph and should contain the following points:

1. Authors' full names followed by current positions;
2. Education background including institution information and year of graduation (type and level of degree received);
3. Work experience;
4. Current and previous research interests;
5. Memberships of professional societies and awards received.

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This journal automatically deposits papers to PubMed Central after publication of an issue. Authors do not need to separately submit their papers through the NIH Manuscript Submission System (NIHMS, **http://nihms.nih.gov/**).

Manuscript Preparation

General Considerations

- **Important note:**
    - Substances without clear ingredients, such as complex prescriptions, crude extracts, and herbal mixtures, are not considered;
    - Papers that only contain clinical trials/data are not acceptable for *IJMS*.
- **Research manuscripts** should comprise:
    - **Front matter**: Title, Author list, Affiliations, Abstract, Keywords.
    - **Research manuscript sections**: Introduction, Results, Discussion, Materials and Methods, Conclusions (optional).
    - **Back matter**: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, **References**.
- **Review manuscripts** should comprise the **front matter**, literature review sections and the **back matter**. The template file can also be used to prepare the front and back matter of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the **PRISMA** guidelines.
- **Case reports** should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.

- **Graphical Abstract:**

  A graphical abstract (GA) is an image that appears alongside the text abstract in the Table of Contents. In addition to summarizing the content, it should represent the topic of the article in an attention-grabbing way. Moreover, it should not be exactly the same as the Figure in the paper or just a simple superposition of several subfigures. Note that the GA must be original and unpublished artwork. Any postage stamps, currency from any country, or trademarked items should not be included in it.

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  The minimum required size for the GA is 560 × 1100 pixels (height × width). The size should be of high quality in order to reproduce well.

- **Acronyms/Abbreviations/Initialisms** should be defined the first time they appear in each of three sections: the abstract; the main text; the first figure or table. When defined for the first time, the acronym/abbreviation/initialism should be added in parentheses after the written-out form.
- **SI Units** (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.
- **Accession numbers** of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Also see the section on **Deposition of Sequences and Expression Data**.
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These sections should appear in all manuscript types

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Please do not include abbreviated or short forms of the title, such as a running title or head. These will be removed by our Editorial Office.

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- *IJMS* can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.
- All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).
- All Figures, Schemes and Tables should have a short explanatory title and caption.
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Supplementary Materials, Data Deposit and Software Source Code

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Data Availability Statements provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study.

Below are suggested Data Availability Statements:

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  The data presented in this study are openly available in [repository name e.g., FigShare] at [**doi**], reference number [reference number].
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- Data available on request due to restrictions eg privacy or ethical
  The data presented in this study are available on request from the corresponding author. The data are not publicly available due to [insert reason here]
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  Restrictions apply to the availability of these data. Data was obtained from [third party] and are available [from the authors / at URL] with the permission of [third party].
- Data sharing not applicable
  No new data were created or analyzed in this study. Data sharing is not applicable to this article.

- Data is contained within the article or supplementary material
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Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of the peer-review process. Any file format is acceptable; however, we recommend that common, non-proprietary formats are used where possible. For more information on supplementary materials, please refer to **https://www.mdpi.com/authors/layout#_bookmark83**.

References in Supplementary Files

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Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult **databib.org** or **re3data.org**. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal **Data** also accepts submissions of data set papers.

Deposition of Sequences and Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be

included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

- *New nucleic acid sequences* must be deposited into an acceptable repository such as **GenBank**, **EMBL**, or **DDBJ**. Sequences should be submitted to only one database.
- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the **GEO database** or in the NCBI's **Sequence Read Archive (SRA)**.
- *New microarray data* must be deposited either in the **GEO** or the **ArrayExpress** databases.The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.
- *New protein sequences* obtained by protein sequencing must be submitted to UniProt (submission tool **SPIN**). Annotated protein structure and its reference sequence must be submitted to **RCSB of Protein Data Bank**.

All sequence names and the accession numbers provided by the databases must be provided in the Materials and Methods section of the article.

Deposition of Proteomics Data

Methods used to generate the proteomics data should be described in detail and we encourage authors to adhere to the "**Minimum Information About a Proteomics Experiment**". All generated mass spectrometry raw data must be deposited in the appropriate public database such as **ProteomeXchange**, **PRIDE** or **jPOST**. At the time of submission, please include all relevant information in the materials and methods section, such as repository where the data was submitted and link, data set identifier, username and password needed to access the data.

Research and Publication Ethics

Research Ethics

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (**https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/**), revised in 2013. According to point 23 of this declaration, an approval from the local institutional review board (IRB) or other appropriate ethics committee must be obtained before undertaking the research to confirm the study meets national and international guidelines. As a minimum, a statement including the project identification code, date of approval, and name of the ethics committee or institutional review board must be stated in Section 'Institutional Review Board Statement' of the article.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study. If ethical approval is not required, authors must either provide an exemption from the ethics committee or are encouraged to cite the local or national legislation that indicates ethics approval is not required for this type of study. Where a study has been granted exemption, the name of the ethics committee which provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation regarding why ethical approval was not required.

A written informed consent for publication must be obtained from participating patients. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of participants' faces that show a particular symptom). Patients' initials or other personal identifiers must not appear in any images. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent for publication from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions. A **template permission form** is available to download. A blank version of the form used to obtain permission (without the patient names or signature) must be uploaded with your submission. Editors reserve the right to reject any submission that does not meet these requirements.

You may refer to our sample form and provide an appropriate form after consulting with your affiliated institution. For the purposes of publishing in MDPI journals, a consent, permission, or release form should include unlimited permission for publication in all formats (including print, electronic, and online), in sublicensed and reprinted versions (including translations and derived works), and in other works and products under open access license. To respect patients' and any other individual's privacy, please do not send signed forms. The journal reserves the right to ask authors to provide signed forms if necessary.

If the study reports research involving vulnerable groups, an additional check may be performed. The submitted manuscript will be scrutinized by the editorial office and upon request, documentary evidence (blank consent forms and any related discussion documents from the ethics board) must be supplied. Additionally, when studies describe groups by race, ethnicity, gender, disability, disease, etc., explanation regarding why such categorization was needed must be clearly stated in the article.

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs [1]':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and

- Refinement of experimental conditions and procedures to minimize the harm to animals.

Authors must include details on housing, husbandry and pain management in their manuscript.

For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [2], American Association for Laboratory Animal Science [3] or European Animal Research Association [4].

If national legislation requires it, studies involving vertebrates or higher invertebrates must only be carried out after obtaining approval from the appropriate ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be stated in Section 'Institutional Review Board Statement'. Research procedures must be carried out in accordance with national and institutional regulations. Statements on animal welfare should confirm that the study complied with all relevant legislation. Clinical studies involving animals and interventions outside of routine care require ethics committee oversight as per the American Veterinary Medical Association. If the study involved client-owned animals, informed client consent must be obtained and certified in the manuscript report of the research. Owners must be fully informed if there are any risks associated with the procedures and that the research will be published. If available, a high standard of veterinary care must be provided. Authors are responsible for correctness of the statements provided in the manuscript.

If ethical approval is not required by national laws, authors must provide an exemption from the ethics committee, if one is available. Where a study has been granted exemption, the name of the ethics committee that provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation on why the ethical approval was not required.

If no animal ethics committee is available to review applications, authors should be aware that the ethics of their research will be evaluated by reviewers and editors. Authors should provide a statement justifying the work from an ethical perspective, using the same utilitarian framework that is used by ethics committees. Authors may be asked to provide this even if they have received ethical approval.

MDPI endorses the ARRIVE guidelines (**arriveguidelines.org/**) for reporting experiments using live animals. Authors and reviewers must use the ARRIVE guidelines as a checklist, which can be found at **https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20Compliance%20Questionnaire.pdf**. Editors reserve the right to ask for the checklist and to reject submissions that do not adhere to these guidelines, to reject submissions based on ethical or animal welfare concerns or if the procedure described does not appear to be justified by the value of the work presented.

1. NSW Department of Primary Industries and Animal Research Review Panel. Three Rs. Available online: **https://www.animalethics.org.au/three-rs**
2. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. Available online: **https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388535/CoPanimalsWeb.pdf**

3. American Association for Laboratory Animal Science. The Scientific Basis for Regulation of Animal Care and Use. Available online: **https://www.aalas.org/about-aalas/position-papers/scientific-basis-for-regulation-of-animal-care-and-use**

4. European Animal Research Association. EU regulations on animal research. Available online: **https://www.eara.eu/animal-research-law**

Research Involving Cell Lines

Methods sections for submissions reporting on research with cell lines should state the origin of any cell lines. For established cell lines the provenance should be stated and references must also be given to either a published paper or to a commercial source. If previously unpublished *de novo* cell lines were used, including those gifted from another laboratory, details of institutional review board or ethics committee approval must be given, and confirmation of written informed consent must be provided if the line is of human origin.

An example of Ethical Statements:

The HCT116 cell line was obtained from XXXX. The $MLH1^{+}$ cell line was provided by XXXXX, Ltd. The DLD-1 cell line was obtained from Dr. XXXX. The DR-GFP and SA-GFP reporter plasmids were obtained from Dr. XXX and the Rad51K133A expression vector was obtained from Dr. XXXX.

Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the **Convention on Biological Diversity** and the **Convention on the Trade in Endangered Species of Wild Fauna and Flora**.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana, Nicotiana benthamiana, Oryza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from ‘Crown Mix’ (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX , institute, city, country).

Clinical Trials Registration

Registration

MDPI follows the International Committee of Medical Journal Editors (ICMJE) **guidelines** which require and recommend registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication.

Purely observational studies do not require registration. A clinical trial not only refers to studies that take place in a hospital or involve pharmaceuticals, but also refer to all studies which involve participant randomization and group classification in the context of the intervention under assessment.

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register and cite a reference to the registration in the Methods section. Suitable databases include **clinicaltrials.gov**, **the EU Clinical Trials Register** and those listed by the World Health Organisation **International Clinical Trials Registry Platform**.

Approval to conduct a study from an independent local, regional, or national review body is not equivalent to prospective clinical trial registration. MDPI reserves the right to decline any paper without trial registration for further peer-review. However, if the study protocol has been published before the enrolment, the registration can be waived with correct citation of the published protocol.

CONSORT Statement

MDPI requires a completed CONSORT 2010 **checklist** and **flow diagram** as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (**http://www.consort-statement.org**) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist.

Sex and Gender in Research

We encourage our authors to follow the **'Sex and Gender Equity in Research – SAGER – guidelines'** and to include sex and gender considerations where relevant. Authors should use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Article titles and/or abstracts should indicate clearly what sex(es) the study applies to. Authors should also describe in the background, whether sex and/or gender differences may be expected; report how sex and/or gender were accounted for in the design of the study; provide disaggregated data by sex and/or gender, where appropriate; and discuss respective results. If a sex and/or gender analysis was not conducted, the rationale should be given in the Discussion. We suggest that our authors consult the full **guidelines** before submission.

Borders and Territories

Potential disputes over borders and territories may have particular relevance for authors in describing their research or in an author or editor correspondence address, and should be respected. Content decisions are an editorial matter and where there is a

potential or perceived dispute or complaint, the editorial team will attempt to find a resolution that satisfies parties involved.

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Publication Ethics Statement

IJMS is a member of the Committee on Publication Ethics (**COPE**). We fully adhere to its **Code of Conduct** and to its **Best Practice Guidelines**.

The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *IJMS* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *IJMS* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
- Authors should accurately present their research findings and include an objective discussion of the significance of their findings.
- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.
- Simultaneous submission of manuscripts to more than one journal is not tolerated.
- The journal accepts exact translations of previously published work. All submissions of translations must conform with our **policies on translations**.
- If errors and inaccuracies are found by the authors after publication of their paper, they need to be promptly communicated to the editors of this journal so that appropriate actions can be taken. Please refer to our **policy regarding Updating Published Papers**.
- Your manuscript should not contain any information that has already been published. If you include already published figures or images, please obtain the necessary permission from the copyright holder to publish under the CC-BY license. For further information, see the **Rights and Permissions** page.
- Plagiarism, data fabrication and image manipulation are not tolerated.
    - **Plagiarism is not acceptable** in *IJMS* submissions.

      Plagiarism includes copying text, ideas, images, or data from another source, even from your own publications, without giving any credit to the original source.

Reuse of text that is copied from another source must be between quotes and the original source must be cited. If a study's design or the manuscript's structure or language has been inspired by previous works, these works must be explicitly cited.

All MDPI submissions are checked for plagiarism using the industry standard software iThenticate. If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, an investigation will take place and action taken in accordance with our policies.

- **Image files must not be manipulated or adjusted in any way** that could lead to misinterpretation of the information provided by the original image.

Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

Citation Policy

Authors should ensure that where material is taken from other sources (including their own published writing) the source is clearly cited and that where appropriate permission is obtained.

Authors should not engage in excessive self-citation of their own work.

Authors should not copy references from other publications if they have not read the cited work.

Authors should not preferentially cite their own or their friends', peers', or institution's publications.

Authors should not cite advertisements or advertorial material.

In accordance with COPE guidelines, we expect that "original wording taken directly from publications by other researchers should appear in quotation marks with the appropriate citations." This condition also applies to an author's own work. COPE have produced a discussion document on **citation manipulation** with recommendations for best practice.

Reviewer Suggestions

During the submission process, please suggest five potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper.

English Corrections

To facilitate proper peer-reviewing of your manuscript, it is essential that it is submitted in grammatically correct English. Advice on some specific language points can be found **here**.

MDPI provides minor English editing by native English speakers for all accepted papers, included in the APC. The APC does not cover extensive English editing. Your paper could be returned to you at the English editing stage of the publication process if extensive editing is required. You may choose to use a paid language-editing service, such as MDPI's **Author Services**, before submitting your paper for publication. If you use an alternative service that provides a confirmation certificate, please send a copy to the Editorial Office. Authors from economically developing countries or nations should consider registration with **AuthorAid**, a global research community that provides networking, mentoring, resources and training for researchers.

Preprints and Conference Papers

IJMS accepts submissions that have previously been made available as preprints provided that they have not undergone peer review. A preprint is a draft version of a paper made available online before submission to a journal.

MDPI operates ***Preprints***, a preprint server to which submitted papers can be uploaded directly after completing journal submission. Note that *Preprints* operates independently of the journal and posting a preprint does not affect the peer review process. Check the *Preprints* **instructions for authors** for further information.

Expanded and high-quality conference papers can be considered as articles if they fulfill the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright of the published conference paper, authors should seek the appropriate permission from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what has been changed compared to the original conference paper. *IJMS* does not publish pilot studies or studies with inadequate statistical power.

Unpublished conference papers that do not meet the above conditions are recommended to be submitted to the **Proceedings Series journals**.

Authorship

MDPI follows the International Committee of Medical Journal Editors (**ICMJE**) guidelines which state that, in order to qualify for authorship of a manuscript, the following criteria should be observed:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgments. More detailed guidance on authorship is given by the **International Council of Medical Journal Editors (ICMJE)**.

Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication. We reserve the right to request confirmation that all authors meet the authorship conditions.

For more details about authorship please check **MDPI ethics website**.

Reviewers Recommendation

Authors can recommend potential reviewers. Journal editors will check to make sure there are no conflicts of interest before contacting those reviewers, and will not consider those with competing interests. Reviewers are asked to declare any conflicts of interest. Authors can also enter the names of potential peer reviewers they wish to exclude from consideration in the peer review of their manuscript, during the initial submission progress. The editorial team will respect these requests so long as this does not interfere with the objective and thorough assessment of the submission.

Editorial Independence

Lack of Interference With Editorial Decisions

Editorial independence is of utmost importance and MDPI does not interfere with editorial decisions. All articles published by MDPI are peer reviewed and assessed by our independent editorial boards, and MDPI staff are not involved in decisions to accept manuscripts. When making an editorial decision, we expect the academic editor to make their decision based only upon:

- The suitability of selected reviewers;
- Adequacy of reviewer comments and author response;
- Overall scientific quality of the paper.

In all of our journals, in every aspect of operation, MDPI policies are informed by the mission to make science and research findings open and accessible as widely and rapidly as possible.

Editors and Editorial Staff as Authors

Editorial staff or editors shall not be involved in processing their own academic work. Submissions authored by editorial staff/editors will be assigned to at least two

independent outside reviewers. Decisions will be made by other Editorial Board Members who do not have a conflict of interest with the author. Journal staff are not involved in the processing of their own work submitted to any MDPI journals.

Conflicts of Interest

According to The International Committee of Medical Journal Editors, "Authors should avoid entering into agreements with study sponsors, both for-profit and non-profit, that interfere with authors' access to all of the study's data or that interfere with their ability to analyze and interpret the data and to prepare and publish manuscripts independently when and where they choose."

All authors must disclose all relationships or interests that could inappropriately influence or bias their work. Examples of potential conflicts of interest include but are not limited to financial interests (such as membership, employment, consultancies, stocks/shares ownership, honoraria, grants or other funding, paid expert testimonies and patent-licensing arrangements) and non-financial interests (such as personal or professional relationships, affiliations, personal beliefs).

Authors can disclose potential conflicts of interest via the online submission system during the submission process. Declarations regarding conflicts of interest can also be collected via the **MDPI disclosure form**. The corresponding author must include a summary statement in the manuscript in a separate section "Conflicts of Interest" placed just before the reference list. The statement should reflect all the collected potential conflicts of interest disclosures in the form.

See below for examples of disclosures:

Conflicts of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stocks in Company Y. Author C has been involved as a consultant and expert witness in Company Z. Author D is the inventor of patent X.

If no conflicts exist, the authors should state:

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

Editorial Procedures and Peer-Review

Initial Checks

All submitted manuscripts received by the Editorial Office will be checked by a professional in-house *Managing Editor* to determine whether they are properly prepared and whether they follow the ethical policies of the journal, including those for human and animal experimentation. Manuscripts that do not fit the journal's ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission. After these checks, the *Managing Editor* will consult the journals' *Editor-in-Chief* or *Associate Editors* to determine whether the manuscript fits the scope of the journal and whether it is scientifically sound. No judgment on the potential impact of the work will be made at this stage. Reject decisions at this stage will be verified by the *Editor-in-Chief*.

Peer-Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. A single-blind review is applied, where authors' identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

In the case of regular submissions, in-house assistant editors will invite experts, including recommendations by an academic editor. These experts may also include *Editorial Board Members* and Guest Editors of the journal. Potential reviewers suggested by the authors may also be considered. Reviewers should not have published with any of the co-authors during the past three years and should not currently work or collaborate with any of the institutions of the co-authors of the submitted manuscript.

Optional Open Peer-Review

The journal operates optional open peer-review: *Authors are given the option for all review reports and editorial decisions to be published alongside their manuscript. In addition, reviewers can sign their review, i.e., identify themselves in the published review reports.* Authors can alter their choice for open review at any time before publication, but once the paper has been published changes will only be made at the discretion of the *Publisher* and *Editor-in-Chief*. We encourage authors to take advantage of this opportunity as proof of the rigorous process employed in publishing their research. To guarantee impartial refereeing, the names of referees will be revealed only if the referees agree to do so, and after a paper has been accepted for publication.

Editorial Decision and Revision

All the articles, reviews and communications published in MDPI journals go through the peer-review process and receive at least two reviews. The in-house editor will communicate the decision of the academic editor, which will be one of the following:

- *Accept after Minor Revisions*:
  The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.
- *Reconsider after Major Revisions*:
  The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. A maximum of two rounds of major revision per manuscript is normally provided. Authors will be asked to resubmit the revised paper within a suitable time frame, and the revised version will be returned to the reviewer for further comments. If the required revision time is estimated to be longer than 2 months, we will recommend that authors withdraw their manuscript before resubmitting so as to avoid unnecessary time pressure and to ensure that all manuscripts are sufficiently revised.
- *Reject and Encourage Resubmission*:
  If additional experiments are needed to support the conclusions, the manuscript will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.
- *Reject*:
  The article has serious flaws, and/or makes no original significant contribution. No offer of resubmission to the journal is provided.

All reviewer comments should be responded to in a point-by-point fashion. Where the authors disagree with a reviewer, they must provide a clear response.

Author Appeals

Authors may appeal a rejection by sending an e-mail to the Editorial Office of the journal. The appeal must provide a detailed justification, including point-by-point responses to the reviewers' and/or Editor's comments using an **appeal form**. Appeals can only be submitted following a "reject and decline resubmission" decision and should be submitted within three months from the decision date. Failure to meet these criteria will result in the appeal not being considered further. The *Managing Editor* will forward the manuscript and related information (including the identities of the referees) to a designated *Editorial Board Member*. The Academic Editor being consulted will be asked to provide an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. This decision will then be validated by the *Editor-in-Chief*. A reject decision at this stage is final and cannot be reversed.

Production and Publication

Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, final corrections, pagination, and, publication on the **www.mdpi.com** website.

Promoting Equity, Diversity and Inclusiveness Within MDPI Journals

Our Managing Editors encourage the Editors-in-Chief and Associate Editors to appoint diverse expert Editorial Boards. This is also reflective in our multi-national and inclusive workplace. We are proud to create equal opportunities without regard to gender, ethnicity, sexual orientation, age, religion, or socio-economic status. There is no place for discrimination in our workplace and editors of MDPI journals are to uphold these principles in high regard.

Resource Identification Initiative

To improve the reproducibility of scientific research, the **Resource Identification Initiative** aims to provide unique persistent identifiers for key biological resources, including antibodies, cell lines, model organisms and tools.

We encourage authors to include unique identifiers - RRIDs- provided by the **Resource Identification Portal** in the dedicated section of the manuscript.

To help authors quickly find the correct identifiers for their materials, there is a single **website** where all resource types can be found and a 'cite this' button next to each resource, that contains a proper citation text that should be included in the methods section of the manuscript.