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MODULAÇÃO DO FATOR DE TRANSCRIÇÃO TBX2 COMO NOVA ESTRATÉGIA ANTICÂNCER: ESTUDO BASEADO NA ATIVIDADE BIOLÓGICA DE CROMOMICINA A_5 EM CÉLULAS DE MELANOMA

Tese apresentada ao programa de Pós-graduação em Farmacologia ao Instituto de Ciências Biomédicas da Universidade de São de Paulo para obtenção do título de doutora em Ciências

Área de concentração: Farmacologia
Orientadora: Profa. Dra. Leticia Veras Costa Lotufo
Versão original

São Paulo
2020

Modulação do fator de transcrição TBX2 como nova estratégia anticâncer: estudo baseado na atividade biológica de cromomicina A_5 em células de melanoma. Autora: Bianca Del Bianco Sahm. Orientadora: Leticia Veras Costa Lotufo. Programa de pós-graduação em Farmacologia, ICB-USP.

Resumo

O melanoma é uma neoplasia derivada de melanócitos, que apresenta alta taxa de heterogeneidade, reduzindo a eficácia do tratamento disponível para essa doença. Assim sendo, a busca por novos tratamentos que atuem em alvo moleculares distintos, ainda é necessária na terapia do melanoma. Estudos demonstraram que o fator de transcrição TBX2, conhecido por suas funções na embriogênese, favorece a proliferação e imortalidade de células de melanoma através do escape da senescência, e ainda confere resistência ao tratamento com agentes alquilantes de DNA, sendo considerado um novo alvo anticâncer. Os produtos naturais marinhos compreendem um grande arsenal de moléculas únicas com diversas atividades biológicas, sendo uma importante fonte de compostos com atividade anticâncer. Assim, o presente estudo se propôs a identificar compostos naturais de origem marinha com potencial de ligação ao fator de transcrição TBX2. Nossa estratégia de prospecção foi a utilização de um procedimento de afinidade reversa, no qual a proteína recombinante de TBX2 foi utilizada como isca. No presente estudo nós exploramos a capacidade de bactérias marinhas em produzir compostos bioativos a partir da descrição do isolamento e caracterização de moléculas pertencente à classe das cromomicinas. Posteriormente, as cromomicinas foram testadas frente ao seu potencial de ligação com TBX2. Os resultados evidenciam que cromomicina A_5 (CA_5) potencialmente se liga a este alvo. CA_5 também apresentou forte atividade citotóxica contra uma linhagem de melanoma metastático dependente de TBX2 (IC_{50} 0.8 nM). Ainda, usando modelos geneticamente modificados, onde a expressão de TBX2 foi aumentada ou diminuída, observou-se que a presença deste fator de transcrição altera o perfil de citotoxicidade de CA_5 nestas células. Estudos seguintes de caracterização da atividade anticâncer sugerem que a modulação da transcrição de TBX2 pode ter participação nos efeitos citotóxicos de CA_5 em células de melanoma. Por fim, nós discutimos o atual cenário de diferentes estratégias que objetivam a

modulação de fatores de transcrição como uma importante abordagem na terapia anticâncer.

Palavras-chave: Anticâncer; terapia alvo-direcionada; TBX2; produtos naturais marinhos; melanoma

Modulation of TBX2 transcription factor as a new anticancer strategy: a study based on the biological activity of chromomycin A5 in melanoma cells. Author: Bianca Del Bianco Sahm. Supervisor: Leticia Veras Costa Lotufo. Graduation program in Pharmacology, ICB-USP.

Abstract

Melanoma is a neoplasm derived from melanocytes, with a high rate of heterogeneity that hinders the effectiveness of the treatment currently available for this disease. Therefore, the search for new treatments that act on different molecular targets is still necessary in melanoma therapy. Studies have shown that the transcription factor TBX2, known for its functions in embryogenesis, favors the proliferation and immortality of melanoma cells through the escape of senescence, and still confers resistance to treatment with DNA-alkylating agents, being considered a new anticancer target. Marine natural products represent a large arsenal of unique molecules with diverse biological activities, being an important source of compounds with anticancer activity. Thus, the present study aimed to identify natural compounds of marine origin with potential for binding to the TBX2 transcription factor. Our prospecting strategy was to use a reverse affinity procedure where the TBX2 recombinant protein was used as bait. In the present study we explored the ability of marine bacteria in producing bioactive compounds and describe the isolation and characterization of molecules belonging to the class of chromomycins. Subsequently, chromomycins were tested for their potential to bind with TBX2. The results show that chromomycin A_5 (CA_5) potentially binds to this target. CA_5 also showed strong cytotoxic activity against a TBX2-driven metastatic melanoma cell line (IC_{50} 0.8 nM). Still, using genetically modified models, where the expression of TBX2 was increased or depleted, revealed that the presence of this transcription factor alters the cytotoxicity profile of CA_5 in these cells. Further studies of anticancer characterization suggest that modulation of TBX2 transcription properties may play a role in the cytotoxic effects of CA5 in melanoma cells. Finally, we discuss the current scenario of different strategies that aim the modulation of transcription factors as an important approach in anticancer therapy.

Keywords: Anti-cancer; target-directed therapy; TBX2; Marine natural products; melanoma.

1. Introduction

1.1 Melanoma

Cancer is the name given to a group of more than 100 diseases that have in common the uncontrolled growth of cells that invade tissues and organs. Dividing rapidly, these cells tend to be very aggressive, leading to the formation of malignant tumors that can spread to other parts of the body (INCA, 2018). Risk factors related to the onset of cancer are, in general, linked to harmful disorders to the genetic material, which can occur endogenously (eg aging, genetic predisposition and heredity) or exogenous (eg smoking, viral infections or exposure to ultraviolet radiation - UVR) (WHO, 2018). With the world population on the rise and older, cancer has been highlighted as one of the most important diseases of the 21st century, with an estimated 18.1 million new cases and 9.6 million deaths caused by the disease in 2018 (FERLAY et al., 2019).

Derived from genetic instability, the development of cancer is complex and heterogeneous. Revealing itself as a model analogous to evolutionary Darwinism, where pro-tumor genes (oncogenes) gain dominant function and tumor suppressor genes become recessive, normal cells begin to express carcinogenic characteristics that, in turn, are transmitted by cell replication (HANAHAN; WEINBERG, 2000). Each type of cancer has a "signature", depending on the type of organ or tissue in which it develops, however, Hanahan and Weinberg (2000 e 2011) reviewed a series of biological capabilities acquired by tumor cells that are common to all neoplasms, in order to rationalize the complexity of this disease.

Briefly, and without necessarily following an order, cancer cells exhibit some or all of the following acquired characteristics: (i) sustaining proliferative signals; (ii) evasion of cell growth suppressors; (iii) resistance to cell death; (iv) capacity for replicative immortality; (v) angiogenesis induction; (vi) invasion and metastasis activation; (vii) reprogramming of energy metabolism; (viii) avoidance of autoimmune destruction. The authors also argue that such acquired capacities are orchestrated by genetic instability (occurrence of genetic mutations is necessary), by the inflammatory condition (which helps in the promotion of multiple capacities) and by the tumor microenvironment (recruitment of normal cells that contribute to the acquisition of capabilities) (HANAHAN; WEINBERG, 2000; 2011).

Among the various types of cancer, the melanoma, derived from melanocytes (melanin-producing cells located in the basal layer of the epidermis, in the central nervous system and in the ocular retina), represents an important public health problem due to its high rate of lethality and resistance to treatment (CURTIN et al., 2005; PRADO; SVOBODA; RIGEL, 2019). Cutaneous melanoma, developed from skin epidermal melanocytes, is the most common type that often appears in regions of high exposure to sunlight, such as arms, hands, neck and face. This kind of melanoma can be subdivided according to its morphological appearance in superficial spreading melanoma, nodular, acral lentiginous melanoma, lentigo maligna, and desmoplastic nevoid. Other less frequent types, called non-cutaneous melanoma, can develop in the choroidal layer of the eye, in the meninges, in the genitourinary mucous membranes or in organs from the respiratory or gastrointestinal systems (TSAO et al., 2012).

The main risk factor for the development of cutaneous melanoma is exposure to ultraviolet radiation (UV-R) emitted by the sun or in tanning chambers. Still, some individual characteristics have been described as favorable for the appearance of this cancer, such as having fair skin, tendency to sunburn, inability to tan, family history, age (> 60 years) and immunosuppression (GILCHREST et al., 1999; KHAN; TRAVERS; KEMP, 2018). This type of skin cancer mainly affects the caucasian population inhabiting regions of the tropics, where its incidence is becoming increasingly common with the number of new cases tripled in the last four decades (SIEGEL; MILLER; JEMAL, 2017; OLSEN et al., 2019).

In 2016, cutaneous melanoma was counted as one of the five most prevalent cancers for the population of the United States, where it is stipulated that one out of 24 Americans is at risk to develop the disease during their lifetime (MILLER et al., 2016; PRADO; SVOBODA; RIGEL, 2019). In Australia, despite the advance of prevention programs, melanoma still ranks third place among the most common and deadly cancers in the country (MAHUMUD et al., 2019). Melanoma also significantly affects the European population, which showed an increase in the incidence rate of both invasive and *in situ* disease (SACCHETTO et al, 2018). The lowest incidence rates, however, are found in African or South American countries, where the population is predominantly composed of racial groups with darker pigmentation (SANTOS; SOUZA, 2019). In Brazil, although the incidence is considered low, its rate almost doubled in 15 years (from 2.52 to 4.84/100,000 men and from 1.93 to 3.22/100,000 women), where melanoma was responsible for about 1800 deaths in 2015 (INCA,

2018; MELO et al., 2018). Early diagnosis and excision of melanomas results in a survival rate of more than 5 years in 99% of cases, but inefficient treatment for more advanced cases of the disease is responsible for 80% of skin cancer-related deaths (PITT et al., 2016; WELSH et al., 2016).

Melanocytes play an important photoprotective function against ultraviolet radiation (UVR). These cells, contained in the basal layer of the epidermis, have dendritic extensions which establish contact with several epithelial keratinocytes. The prolongations are important for transfer of the melanin pigment, which is produced by melanocytes as a product of genetic regulation at the cellular and tissue level or by environmental stimulus, such as exposure to UVR (CICHOREK et al., 2013).

UVR is electromagnetic radiation with wavelengths (λ) ranging from 100 - 400 nm. The main source of this type of radiation is the light emitted by the sun that reaches the Earth's atmosphere. It can be subdivided into UVA (λ = 320 - 400 nm), UVB (λ = 280 - 320 nm) and UVC (λ = 100 - 280 nm), and all types are considered carcinogenic in humans (WHO, 2019). The UVR is absorbed by the cells present in the epidermis, reaching deep levels like proteins and DNA. Its carcinogenic ability is mainly due to genetic damage caused in DNA sequences, either directly (damaging the molecular structure) or indirectly (generation of free radicals and modulation of the cellular response to genomic damage - repair system) (KHAN; TRAVERS; KEMP, 2018). Interestingly, the melanin produced by melanocytes is capable of absorbing and dispersing UVR, making it less harmful to epithelial cells. In addition, melanin promotes the elimination of free radicals coupled with oxidative-reduction reactions, promoting photoprotection by increasing degenerative resistance and neutralizing oxidative stress (ORTONNE, 2002; GHOSH; GHOSH, 2017). That said, it is evident that the lack or deficiency in melanin pigment production raises the risk for development of skin cancers, including melanoma.

Indeed, genomic DNA sequences extracted from melanoma samples have revealed a multitude of genetic mutations typically derived from UVR (REF). Typically, UVR induces the formation of photoproducts in genomic DNA, such as cyclobutane pyrimidine dimers (CPDs), which forms cyclobutane rings in the bond between two pyrimidine bases. Cytosine bases, one of the amino acids that compose the base class of pyrimidines (along with thymine and uracil), are commonly stable and rarely undergo spontaneous removal, which would result in possible genetic mutations (FREDERICO; KUNKEL; SHAW, 1990). However, cytosines surrounded by CPDs are much less

stable, resulting in an increased frequency of spontaneous removals (BURGER et al., 2003). Absent cytosines are replaced by uracil which, if not repaired, are copied by DNA polymerase resulting in fixing the transitions C→T/G→A in DNA sequences. Such transitions characterize the "UV signature" of mutations in the genetic code. The large number of "UV signature" mutations found in cutaneous melanomas shows the relevance of UVR as an important trigger for the appearance and development of this type of cancer (KHAN; TRAVERS; KEMP, 2018).

The melanoma development stages are well defined and orchestrated by genetic changes that characterize the progression to malignant (metastatic) melanoma (Figure 2) (PALMIERI et al., 2018; JACKETT; SCOLYER, 2019). Characteristically, the loss of density-dependent regulation in melanocytes results in the appearance of benign nevi, which is small proliferations with low mutation rate that remain stable due to the functionalization of tumor suppressor genes. However, the acquisition of subsequent genetic changes drives the neoplasm to more aggressive stages. Malignant melanoma can be divided into three distinct phases: radial growth phase (RGP), where the tumor is confined to the epidermis, followed by vertical growth phase (VGP), in which cells invade the lower layer of the dermis, and, finally, the metastatic phase, when the tumor cells spread to other organs (MILLER; MIHN, 2006; SHAIN et al, 2015; JACKETT; SCOLYER, 2019). In general, the success of each stage is related to proliferative efficiency and escape from cellular senescence.

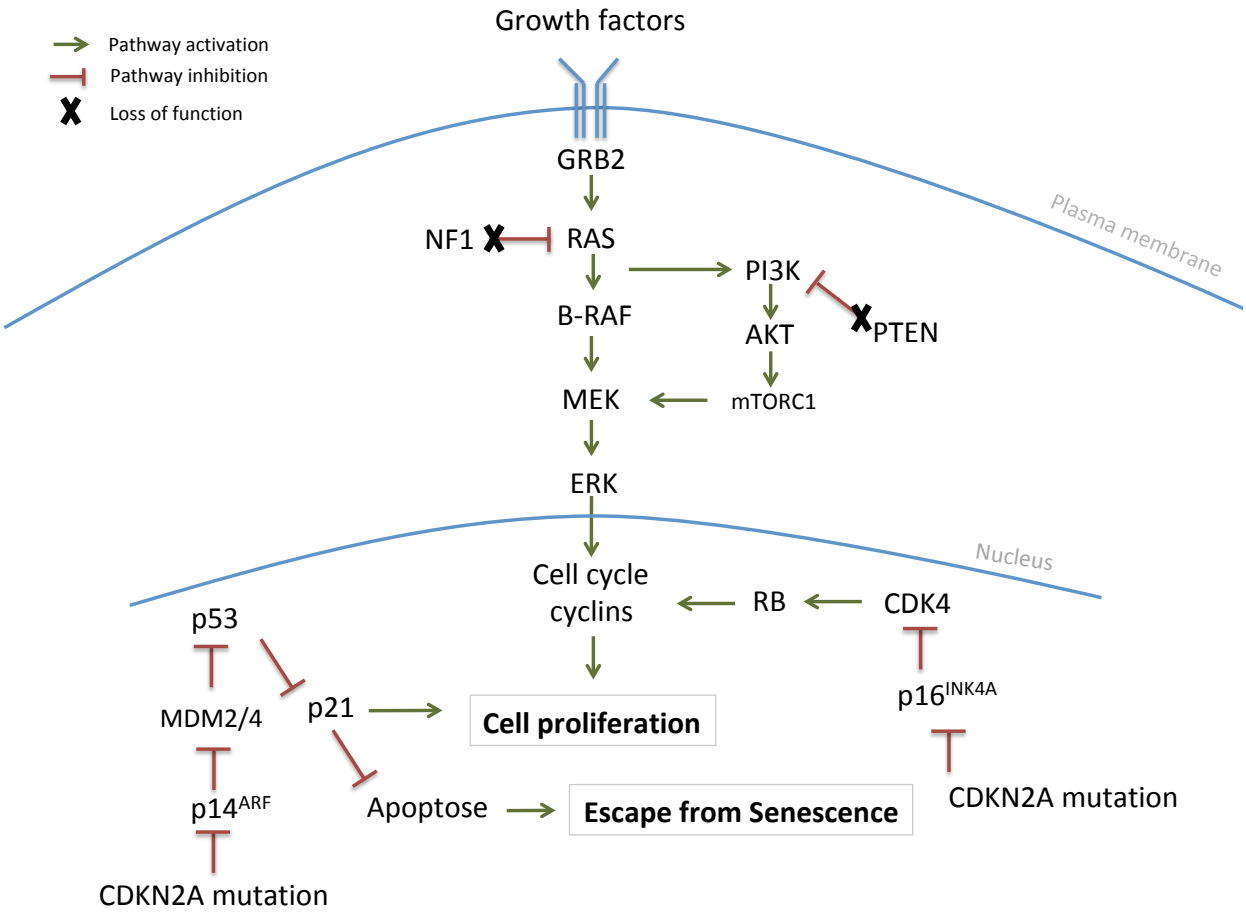
The molecular events associated with the development of melanoma are widely studied and characterized (CURTIN et al., 2005, ALONSO et al., 2004; SHAIN, et al., 2015; PALMIERI et al., 2018; JACKETT; SCOLYER, 2019). Figure 1 below illustrates the most common signaling pathways involved in melanomagenesis. The cancer genetics research network has divided melanoma into 4 subtypes based on the presence of mutations in the BRAF, RAS (N / K / H), NF1 and triple-negative genes (when none of the mutations exist) (TCGA, 2015). More recently, Palmieri et al. (2018) proposed the division into three subtypes: $BRAF^{mut}$, RAS^{mut} and non-$BRAF^{mut}$/non-RAS^{mut}, since they found that the coexistence of mutations in BRAF and RAS is quite uncommon in the same melanoma (PALMIERI et al., 2018).

Usually, the proliferative phenotype occurs due to a gain of function of RAF or RAS kinases, whereas $BRAF^{V600E}$ (substitution of the amino acid valine for glutamic acid in the region of codon 600) and NRAS (mutations in codon Q61) are described as the most frequent mutations (DAVIES et al., 2002). BRAF is active in around 50% of

melanoma tumors, and NRAS mutations in approximately 30% (MILLER; MIHN, 2006; PALMIERI et al., 2018). Both operate the RAS - RAF - MEK1/2 - ERK1/2 pathway, known as MAPK (Mitogen-Activated Protein Kinase), which plays an important role in terms of cell growth, proliferation and survival in melanomas (DAI; MARTINKA; LI, 2005; DAVIES et al., 2002; HAYASHI et al., 2008; LAVOIE; THERRIEN, 2015; MILLER; MIHN, 2006; TSAO et al., 2012). When active, BRAF and NRAS stimulate the constitutive activation of MAPK, which leads to the appearance of proliferative anomalies.

BRAF mutations have been characterized as an important precursor for the appearance of melanoma. Is commonly found in benign nevus, where its oncogenic activation is necessary but not sufficient for the development of malignant disease (PATTON et al., 2005). Moreover, other pathways can also contribute to the pathogenesis and/or resistance to BRAF and MEK inhibition, such as the loss of NF1 (Neurofibromin 1) and activation of the PI3K (Phosphatidylinositol-3-OH kinase) pathway. NF1 is a tumor suppressor that negatively regulates RAS and the loss of its function is found in high mutation burden melanomas, characterized as an UV mutational signature (PHILPOTT et al, 2017; CIRENAJWIS et al., 2017). Constitutive activation of the PI3K pathway occurs in approximately 70% of sporadic melanomas, resulting in loss of function of the tumor suppressor gene PTEN (Phosphatase and Tensin homolog) and/or amplification of AKT3 (serine/threonine kinase 3), which leads to reactivation of MAPK through mTORC1 (CHIAPPETTA et al., 2015; DHAWAN et al., 2002; STAHL et al., 2004; SULLIVAN; FLAHERTY, 2013; TCGA, 2015; VREDEVELD et al., 2012).

Figure 1 - Schematic diagram showing the most common signaling pathways involved in melanomagenesis.



Adapted from John; Cowey, (2015); Cosgaria et al, (2017); Palmieri et al, (2018).

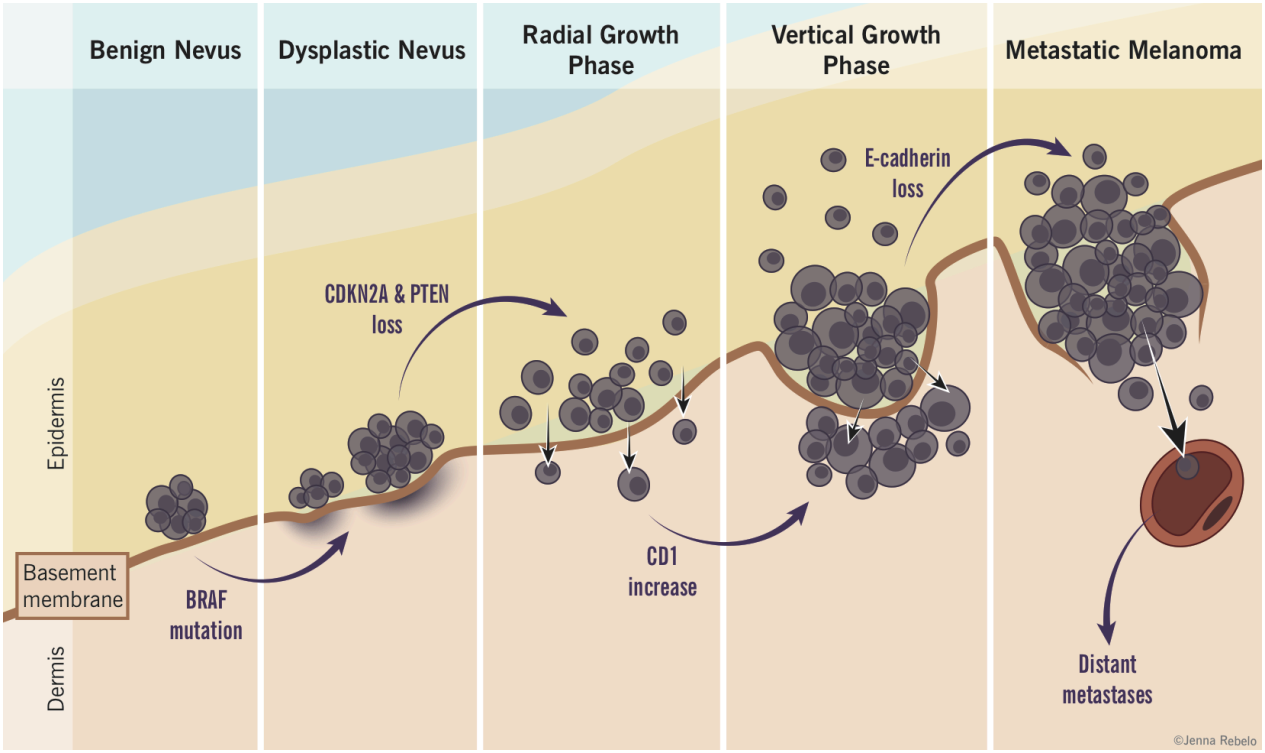
In addition to mutations that promote uncontrolled cell proliferation, another important point acquired by melanoma cells refers to the escape from senescence. Senescence is a term used to define a physiological program of irreversible disruption of cell growth, being an important barrier to malignancy as it prevents the formation of immortal cells (HA; MERLINO; SVIDERSKAYA, 2008; LIU; SHARPLESS, 2012). Cellular senescence can be triggered by telomere shortening, as a consequence of cell division (replicative senescence), or by cellular stress (oncogene-induced senescence). In general, the most important signaling pathways for senescence are $p14^{ARF}$–p53–p21 and $p16^{INK4A}$–RB (ZHANG, 2007). While $p14^{ARF}$ positively regulates the levels of p53 protein (responsible for suppressing DNA replication and activating repair mechanisms or driving the cell to apoptosis) through the inhibition of ubiquitin

ligase MDM2, $p16^{INK4A}$ inhibits CDK4, which directs cell cycle progression through phosphorylation of retinoblastoma protein (RB1), leading, in turn, to the activation of cyclin transcription factors (cell cycle regulation) and thus the expression of S phase genes for cell division (TERZI; IZMIRLI; GOGEBAKAN, 2016). Interferences in any point of the pathways results in cellular escape from DNA repair and senescence.

The loss of tumor suppressor genes is characterized as an important step in the malignant melanoma progression. Mutations in the CDKN2A gene, which encodes the tumor suppressor proteins $p16^{INK4A}$ and $p14^{ARF}$, occur in approximately 40% of familial melanomas (BERWICK et al., 2006; HARLAND et al., 2014; LEACHMAN et al., 2009), followed by mutations in CDK4 (TCGA, 2015). Furthermore, the increase in the expression of the CCND1 gene that encodes CD1 (Cyclin D1), is also an important mutation in the development of melanoma, which when combined with CDK4/6, promotes the positive regulation of cell proliferation through the activation of RB1 (YOUNG et al., 2014; YADAV et al., 2015). Amplifications of TERT (Telomerase Reverse Transcriptase) are also commonly found in melanomas, usually in association with constitutive MAPK activation (BAI et al., 2017). TERT allows the maintenance of telomeres homeostasis by decreasing their progressive shortening during mitosis (HEIDENREICH; KUMAR, 2017).

The transition to the final stage of the disease (invasive metastatic melanoma) also presents important mutational transitions that favor the motility and spread of tumor cells through lymphatic and vascular channels (JACKETT; SCOLYER, 2019). Such events are facilitated by the epithelial-mesenchymal transition (EMT, Epithelial-Mesenchymal Transition), a dynamic and reversible process in which epithelial cells acquire mesenchymal phenotypes, such as increased invasive and migratory capacities (KALLURI; WEINBERG, 2009; LAMOULLI; XU; DERYNCK, 2014). The loss of E-cadherin function, a transmembrane protein that mediate the junction of cell-cell adhesion, is perhaps the critical point for the activation of EMT and transformation of metastatic melanoma (THIERY; SLEEMAN, 2006; KELLER et al, 2013; SUN et al., 2018). The metastatic form adds several mutations that are difficult to control, characterizing it as the terminal phase of the disease, lacking effective treatments available to date. Metastatic cutaneous melanomas tend to colonize lungs, liver, brain and bones (JACKETT; SCOLYER, 2019).

Figure 2 - Illustrative scheme showing the different stages of melanoma development and the main genetic mutations involved for each phase.



BRAF mutations are determinant for the initial proliferative effect leading to the formation of benign nevus. The subsequent loss of tumor suppressor genes, such as CDKN2A and PTEN, favors the transformation from benign to malignant forms. Malignant melanoma then enters the first stage of its development, called the radial growth phase, while remaining retained in the epidermal compartment. In the vertical growth phase, the tumor increases its proliferative capacity, usually by increasing CD1, and initiates colonization of the dermis. The loss of E-cadherin signals the initiation of EMT, which facilitates the metastasis of melanoma to other tissues and organs. Image available at: http://www.pathophys.org/melanoma/melanoma-progression/

There are many molecular processes involved in melanomagenesis, which despite having made great progress in its characterization, there is still much to be unveiled. Several transcription factors have been described as acting directly or assisting tumor capacities acquired in all stages of melanoma (BESCH; BERKING, 2014; ASNAGHI et al., 2015; SEBERG; OTTERLOO; CORNELL, 2017; COHEN-

SOLAL; KAUFMAN; LASFAR, 2018), including T-box transcription factors (WANSLEBEN et al., 2014; PERES; MOWLA; PRINCE, 2015; CHANG et al., 2016).

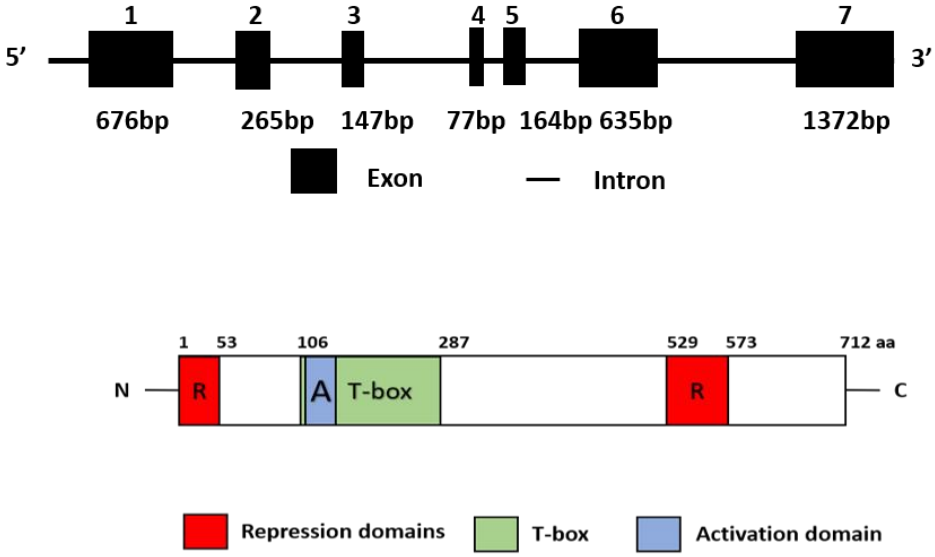
1.2 TBX2 transcription factor

Transcription factors are at the center of the regulatory network that drives gene expression programs in order to promote different biological responses (SPITZ; FURLONG, 2012). The genes belonging to the T-box transcription factors family are important regulators of embryonic development. They are present in all metazoans and are highly conserved along the phylogenetic scale. Based on phylogenetic and expression studies, this family is divided into 5 subfamilies namely: T, Tbx1, Tbx2, Tbx6 and T-brain. All T-box transcription factors are characterized by having a highly conserved DNA binding domain comprised of 180 – 200 amino acid residues (the T-box domain) which recognizes and binds to the core sequence 5'-GGTGTGA-3' (T-element) (SEBĒ-PEDROS & RUIZ- TRILO, 2017), acting as activators or repressors of gene transcription (KAVKA; GREEN, 1997; NAICHE et al., 2005). Because they were initially recognized for their crucial role in embryogenesis, their functions during this process are well described in literature, especially with regard to cell differentiation, proliferation, migration and survival (DOUGLAS; PAPAIOANNOU, 2013; NAICHE et al., 2005).

TBX2 is a member of the subfamily Tbx2, together with TBX4, TBX5 and its closely related homolog TBX3 (AGULNIK et al, 1996). It is encoded by chromosome 17q23, functioning as either a transcriptional activator or repressor. The TBX2 protein is composed by an activation domain prior to the conserved T-box DNA binding domain, and two repression domains, one in the N-terminal with 52 aa residues and the second located in the C-terminal with 529-573 aa residues (figure 3) (CARREIRA, LIU, CODING, 1998; PAXTON et al., 2001; ABRAHAMS et al., 2010; SAKABE et al., 2012). During embryogenesis, the expression of TBX2 is related to the development of heart, kidneys, limb, mammary glands, eyes and lungs (ABRAHAMS, PARKER, PRINCE; 2010; PAPAIOANNOU, 2014; SHEEBA; LOGAN, 2017; WOJAHN et al., 2019). The importance of TBX2 to the embryonic development is highlighted by the fact that disturbances of its expression during embryogenesis can result in abnormal formations. Microdeletions in chromosome 17q23, encompassing the region of TBX2 and TBX4, is described to be related with an unclassified syndrome regarding

developmental disorders such as limb abnormalities, congenital microcephaly, heart defects, pulmonary hypertension, thyroid duct cyst, sensorineural hearing loss and skeletal deformities (BALLIF et al., 2010; NIMMAKAYALU et al., 2011; LAURELL et al., 2013; LIU et al., 2018). Moreover, Tbx2 homozygous mutation in mice results in embryonic death whereas the heterozygous mutant remains viable (WAKKER et al., 2010).

Figure 3 - Schematic representation of TBX2 mRNA and protein.

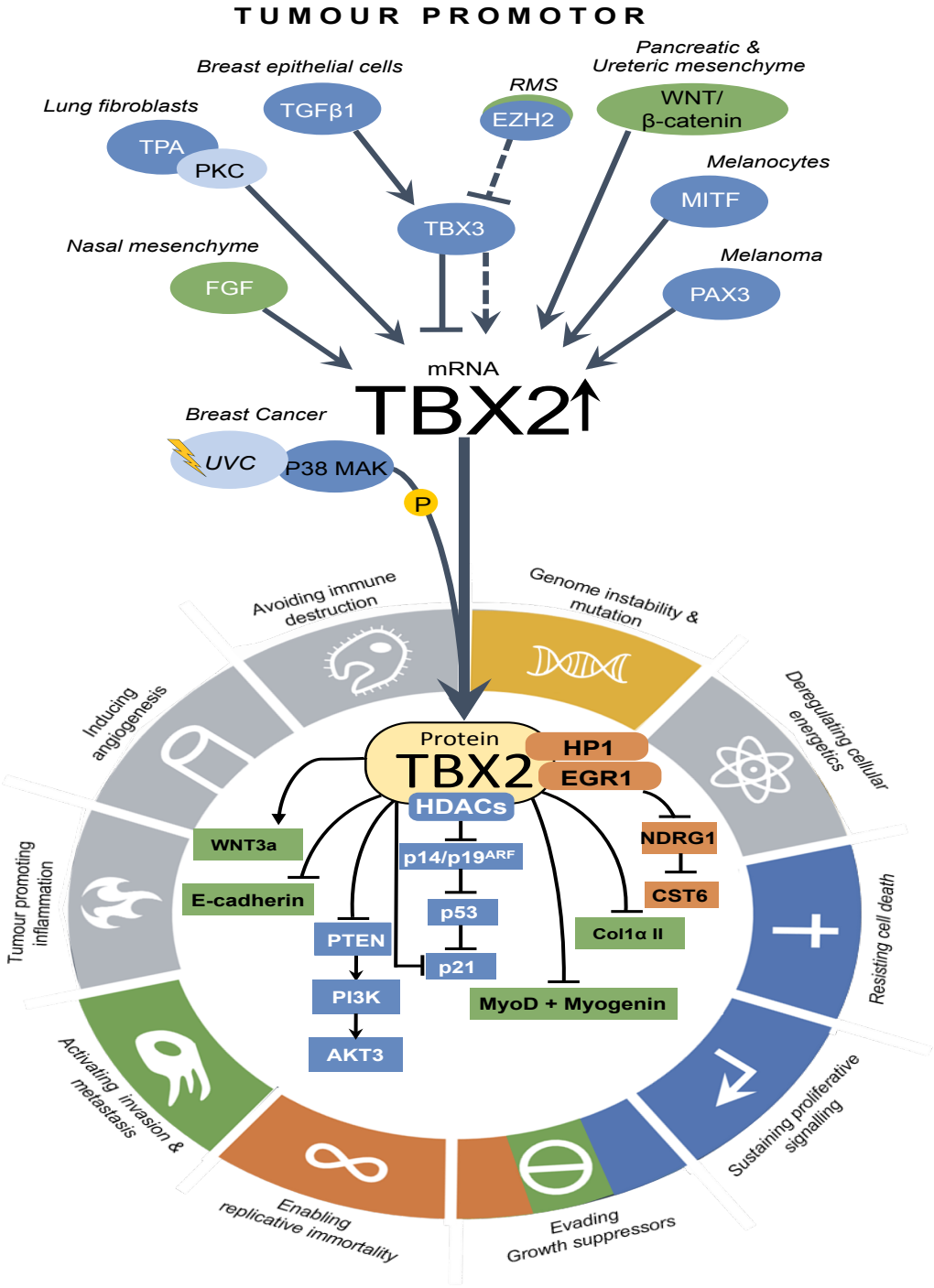


(A) TBX2 mRNA showing seven exons and their respective size. (B) TBX2 protein showing the activation domain (blue), the repression domains (red) and the T-box DNA binding domain (green).

The carcinogenesis process shares some characteristics similar to those that occur during embryonic development such as phenotypic plasticity, proliferative capacity and invasion. Thus said, it is expected that important developmental regulators, including members of the T-box family, are involved in carcinogenesis (BEN-PORATH et al., 2008; BRIEGEL, 2006). In fact, several T-box factors have been described to play a role in tumor promotion or tumor suppression functions (PAPAIOANNOU, 2014; WANSLEBEN et al., 2014; CHANG et al., 2016), with the main collected evidences referring mainly to TBX2 and TBX3 (LINGBEEK; JACOBS; LOHUIZEN, 2002; LU et al., 2010; PERES et al, 2010; DOUGLAS; PAPAIOANNOU., 2013; KHAN et al., 2019).

There is strong evidence to suggest that TBX2 is implicated in key carcinogenesis processes such as tumor cell immortality and proliferation, tumorigenesis, invasion and metastasis through EMT process, and resistance to anticancer chemotherapeutics (JACOBS et al., 2000; PRINCE et al 2004; DAVIS et al., 2008; ABRAHAMS et al., 2010; WANSLEBEN et al., 2013; CHANG et al., 2016). Figure 4 below summarizes the signaling pathways involved in TBX2-driven carcinogenesis processes disclosed so far (Figure 4). It should be noted that different signaling pathways are describe for each type of cancer, and this is not surprising if we consider the high rate of heterogeneity involved in carcinogenesis. Nevertheless, TBX2 seems to be triggered in order to execute its suppression/activation functions over important tumor regulators, adding advantages for tumor progression. The overexpression of TBX2 was initially detected in breast tumors (JACOBS et al., 2000; REDMOND et al., 2010), where its high expression is correlated with a poor prognosis (WANG et al 2012). Currently, TBX2 is known to be overexpressed with tumorigenic functions in a set of cancers including breast (JACOBS et al., 2000; REDMOND et al., 2010) and prostate cancer (DU et al., 2017; NANDANA et al., 2017), nasopharyngeal carcinomas (LV et al., 2017), rhabdomyosarcomas (ZHU et al., 2014), gastric cancer (YU et al., 2015), pancreatic cancer (DUO et al., 2009) and colorectal cancer (HAN et al., 2013).

Figure 4 - Illustrative scheme showing the main signaling pathways described for TBX2 in different cancer cells as tumor promoter.



Scheme elaborated and gently provided by Msc. Mihlali Mlaza, UCT, South Africa.

TBX2 also occurs in several subtypes of melanomas (CARREIRA; LIU; GODING, 2000; VANCE et al., 2005), and a microarray study revealed that the

average expression of TBX2 is significantly higher in melanomas than in other types of tumors (WANG et al., 2004). Moreover, a meta-analysis using data provided by Badal and collaborators (2017), where the transcriptome changes in benign states and late-stages of melanoma tumors were examined, revealing that TBX2 expression is significantly higher in samples from melanoma malignant stages (BADAL et al., 2017) (figure S1 - Supplementary material).

The occurrence of overexpressed TBX2 in melanoma subtypes raised the question of how this gene can contribute to melanomagenesis through promoting the cell's escape from senescence. Studies have shown that TBX2 functions as an anti-senescence factor in human and rodent melanoma cell lines (PRINCE et al., 2004; VANCE et al., 2005; PERES et al., 2010). Previously, Prince et al. (2004) and Vance et al. (2005) demonstrated that Tbx2 in cells of murine melanoma of the B16 lineage, led to the suppression of senescence by direct binding and repressing the $p21^{WAF1}$ cyclin-dependent kinase inhibitor gene, through recruitment of HDAC1 (Histone Deacetylase 1) for the p21 promoter (PRINCE et al., 2004; VANCE et al, 2005). Later, a sequential study carried out by the same group, showed that RB1, when activated, binds to Tbx2 and increases its repression on p21 (VANCE et al., 2010). Following the previous results, Peres et al. (2010) demonstrated that silencing the TBX2 gene in VGP melanoma cells induced several senescence characteristics as well as reduced its proliferation. In a metastatic melanoma cell line, knocking down TBX2 diminished the proliferation rate, but it did not appear to interfere with cell migration abilities (PERES et al., 2010).

Another important function assigned to TBX2 concerns its role in resistance to DNA-alkylating chemotherapeutic agents. It was first demonstrated that the ectopic expression of TBX2 resulted in genetically unstable polyploidy cells with increased resistance to cisplatin treatment (DAVIS et al., 2008). In 2009, a study found that the expression of TBX2 in SW13 adrenocortical carcinoma cells promotes doxorubicin and UV irradiation resistance (ISMAIL; BATEMAN, 2009). More recently, Tasaka et al (2018) reported the inverse association of TBX2 expression levels with platinum-sensitivity in patients with ovarian serous carcinoma (TASAKA et al., 2018).

In an attempt to identify whether the endogenous TBX2 overexpression is associated with DNA-damaging chemotherapeutics resistance, Wansleben et al. (2013) silenced TBX2 in cisplatin-resistant breast carcinoma and melanoma cell lines.

It was demonstrated that the lack of TBX2 sensitized the cells to cisplatin by disrupting the ATM-CHK2-p53 signaling pathway, and that it switched the increased S-phase cell cycle arrest, first found for cisplatin resistance condition, to robust G2/M arrest. The authors concluded that knocking down TBX2 prevented the DNA repair cascade ATM-CHK2-p53 to operate, which results in the TBX2-deficient cells undergoing mitotic catastrophe after entering mitosis with damaged DNA (WANSLEBEN et al., 2013).

Genomic instability is one of the key features of a transformed cell that often culminates in mitotic defects as a result of failure in execution of a proper cell division. Bypassing relevant cell cycle checkpoints, which could interrupt the replication of such mitotic defects, and investing efficient DNA repair systems, are somatic key features found in tumorigenic cells (NIGG, 2001; DAVIS et al., 2008). The effective response of certain anti-cancer chemotherapeutics can be compromised by these modified systems found in cancer cells. To conclude, the findings mentioned above clearly indicate that TBX2 plays important anti-senescence and pro-proliferative roles during melanomagenesis, and that its expression is also important for DNA-damaging resistance in this type of cancer. That said, the inhibition of TBX2 functions could result in diminishing the proliferation of melanoma cells, in addition to increasing sensibility of the malignant cell to the action of DNA alkylating agents, which configures TBX2 as a new anticancer-target for the treatment of melanoma (LU et., 2010; WANSLEBEN et al., 2013).

1.3 Melanoma Therapy

Until a few years ago, typical treatments for melanomas included alkylates drugs such as dacarbazine and temozolomide, which had low efficacy and no improvement in patient overall survival (JOHN; COWEY, 2015). Additionally, immunotherapy-using cytokines such as interferon and interleukin-2 (IL-2) have not respond as expected with high levels of toxicity (ATKINS et al., 1999). In this therapeutic scenario, the average survival rate for patients with metastatic melanoma was between 6 to 8 months after diagnosis, and no new agent had been able to improve this outlook in subsequent clinical trials (MANOLA et al., 2000).

It was from 2011 that a new generation of chemotherapeutics for metastatic melanoma has emerged, with 8 new agents approved by the FDA-USA (Agency for Food and Drug Administration in the USA) in the following 4 years (KNACKSTEDT et

al., 2018; MISHRA et al., 2018). The new therapeutic options have had positive impacts in the response rate and improvement in survival for patients with all stages of melanoma (Table 1). Two main strands were developed: target-driven treatments (COSGAREA et al., 2017) and immunotherapy (RODRÍGUEZ-CERDEIRA et al., 2017).

Vemurafenib and dabrafenib represents the first in the class of drugs for targeted-therapy in the treatment of melanomas, being potent $BRAF^{V600E}$ inhibitors (DAVIES et al., 2002; MENZIES; LONG; MURALI, 2012). The first results of therapy with $BRAF^{V600E}$ inhibitors revealed a rapid response in around 80% of treated patients, increasing the average survival of patients in up to 20 months (HAUSCHILD et al., 2012; MCARTHUR et al., 2014). Despite being promising, soon severe adverse effects such as the appearance of keratoacanthomas and squamous cell carcinoma, in addition to the high rate of recurrence of the disease, limited the use of BRAF inhibitors as monotherapy.

In general, resistance to target-driven treatment involves the re-activation of MAPK pathway through other pathways, resulting in renewed cell proliferation and tumor growth of previously suppressed metastases (JOHANNESSEN et al., 2010). Currently, some combinatorial therapies have been applied in order to circumvent the mechanisms of resistance for target-directed treatment in melanomas, such as the synergistic use of MEK (Mitogen-activated protein Kinase) inhibitors trametinib and cobimetinib (COSGAREA et al., 2017; KAROULIA; GAVATHIOTIS; POULIKAKOS, 2017), however, the definitive success of the therapy has not yet been achieved.

Agents targeting the immune system have also been actively investigated within the aim of improving efficacy and reducing toxicity in melanoma treatments. The greatest advances in this field have been achieved using drugs that block the inhibitory control machinery responsible for the regulation of the immune system, such as PD-1 (Programmed Death 1), and CTLA-4 (Cytotoxic T-Limphocyte-Associeated protein 4). The strategy applied by this therapy involves the inactivation of receptors for cytotoxic activation of T lymphocyte cells, ensuring that they are triggered against the tumor, which, in turns, result in increased patient antitumor immunity (SHARMA; ALLISON, 2015). Denominated as *immune checkpoint inhibitors*, ipilimumab (anti-CTLA-4), nivolumab and pembrolizumab (both anti-PD-1), were approved as monotherapy or combinatorial therapy for the treatment of advanced melanoma due to the good results regarding the survival rate.

Table 1. Therapeutic agents approved by the FDA / ANVISA, from 2011, for the treatment of melanoma.

Agent *(comercial name)*	**Class**	**Mechanism of action**	**Approved year (FDA/ANVISA)**
Vemurafenib *(Zelboraf®)*	Target-driven molecule	$BRAF^{V600E}$ inhibitor	2011/2012
Dabrafenib *(Tafinlar®)*		$BRAF^{V600E/K}$ inhibitor	2013/2016
Trametinib *(Meknist®)*		MEK inhibitor	2013/2016
Cobimetinib *(Cotellic®)*		MEK inhibitor	2015/2016
Ipilimumab *(Yervoy®)*	Immune system checkpoint inhibitors	Anti-CTLA-4	2011/2012
Pembrolizumab *(Keytruda®)*		Anti-PD-1	2014/2016
Nivolumab *(Opdivo®)*		Anti-PD-1	2014/2016

Adapted from (KAROULIA; GAVATHIOTIS; POULIKAKOS, 2017; SHARMA; ALLISON, 2015; KNACKSTEDT et al., 2018; MISHRA et al., 2018).

In addition, topical immunotherapy for cutaneous melanoma is currently being explored. Imiquimod is an agonist of toll-like receptors (TLR) 7 and 8 and. This immunomodulatory drug upregulates the release of cytokines (e.g. IL-6, IFN-α and TNF-α) and the nitric oxide secretion from macrophages (KAMATHY et al., 2018), stimulating the immune system cancer attack. There are good evidences showing that imiquimod is effective as adjuvant therapy after surgical excision in first-stages melanoma, but its action in metastatic stages is still not satisfactory (KNACKSTEDT et al., 2018). A more recent approach for the immunological treatment of melanomas is the intratumoral injection of oncolytic viruses. Despite being yet poorly developed, in 2015 the FDA approved the Talimogene laherparepvec (T-VEC) for the treatment of

unresectable melanoma, evidencing improved median overall survival of patients with intermediate melanoma stages (ANDTBACKA et al., 2015; KNACKSTEDT et al., 2018). T-VEC is a *Herpes simplex* virus type-1 designed to selective replicate within tumors and deliver oncolytic immunotherapy by producing granulocyte macrophage colony-stimulating factor (GM-CSF) to enhance systemic antitumor immune response (ANDTBACKA et al., 2015).

Despite the actual existence of different options, the search for effective treatment against metastatic melanoma continues. Different approaches such as the combination of other immunotherapies, development of new formulations using nanoparticles and the elucidation of new molecular targets therapeutically relevant to the disease can further expand these results (JOHN; COWEY, 2015; MISHRA et al., 2018).

1.4 Marine Natural Products and cancer

Natural products play a fundamental role in the discovery of new drugs as they comprise a diversity of chemical structures, sometimes highly complex, with relevant biological activities which have found therapeutic application for several diseases (CARLSON, 2010; HARVEY, 2008). The great value of natural compounds in the field of new drugs discovery is particularly evident in the case of anti-cancer agents, where, of the more than 170 molecules that came into clinical use between years 1940 and 2014, 75% are classified as non-synthetic, and approximately 50% are extrinsic natural products or directly derived from them (CRAGG; NEWMAN, 2016).

In addition to the classic terrestrial sources for natural products, such as plants, bacteria and fungi, a field that is gaining prominence is the research evolving natural products from marine origin (MOLINSKI et al., 2009; NEWMAN; CRAGG, 2016). Seas and oceans occupy more than 2/3 of the Earth's surface, where diverse and extreme ecosystems are inserted. Moreover, the marine environment host 34 of the 36 phyla representing living organisms, including some strictly marine phyla (ARRIETA; ARNAUD-HAOND; DUARTE, 2010; FENICAL, 2006; RAMIREZ-LLODRA et al., 2010). In this scenario, for at least 60 years researchers have been exploring the pharmacological potential of secondary metabolites from marine organisms, motivated by the size of the oceans and the still unknown level of biodiversity in this environment (GLASER; MAYER, 2009). Nowadays, it is well established that the marine environment is a valuable source of natural products that presents great variability of

chemical structures with promising bioactivities useful as therapeutic agents (BLUNT et al., 2018; JIMENEZ; WILKE; LOTUFO, 2018; SHINDE et al., 2019)

To date, 10 marine-based pharmaceuticals have been approved for clinical use and there are a considerable number of substances undergoing pre-clinical and clinical tests for the treatment of various diseases (https://www.midwestern.edu/departments/marinepharmacology/clinical-pipeline.xml). Also, the great number of new marine molecules patents, filed between 2015 and 2018, clearly highlights the value of these chemicals as sources of new therapeutic agents (SHINDE et al., 2019). Among those marine-derived drugs in the clinical field, seven of them are prescribed for cancer treatment: cytarabine (Cytosar®), trabectedin (Yondelis®), eribulin mesylate (Halaven®), plitidepsin (Aplidin®) and the antibodies conjugates (ADC): brentuximab vedotin (Acentris®), enfortumab vedotin-ejfv (PADCEV™) and Polatuzumamu vedotin (Polivy™) (JIMENEZ et al., 2020; https://www.midwestern.edu/departments/marinepharmacology/clinical-pipeline.xml).

Cytarabine (Cytosar®) was the first approved for clinical use in 1969. This molecule is a synthetic arabinose-nucleoside, which was inspired by previously isolated natural products derived from the caribbean marine sponge *Tectithethya crypta* (BERGMANN; FEENEY, 1951; MURPHY; YEE, 2017). Since this molecule mimics the cytosine nucleotide, its mechanism of action involves the incorporation of cytarabine, into the DNA, in the place of cytosine. The presence of arabinose coupled to cytarabine, instead of deoxyribose, prevents the elongation of DNA strains, thus blocking the process of DNA synthesis (NEWMANN; CRAGG, 2014; 2016). This drug inaugurated the concept of antimetabolites in cancer Therapy. Eribulin mesylate (Halaven®) is a mesylate salt analog to halichondrin B, a macrocyclic polyether natural molecule previously isolated from another marine sponge *Halicondriha okadai*. It is a potent antimitotic compound that acts as an inhibitor of microtubule dynamics by targeting and immobilizing the globular protein tubulin. The result is an irreversible mitosis blockage which leads to cell death by apoptosis (JORDAN et al., 2005; JIMENEZ; WILKE; COSTA-LOTUFO, 2018).

Presumably, the most interesting marine compound is trabectedin (Yondelis®), which has been shown to play an unique and multivariate anticancer mechanism of action. Trabectedin is a natural product previously isolated from the ascidian tunicate marine invertebrate *Ecteinascia turbinata* (CUEVAS; FRANCESCH, 2009), currently produced in a semi-synthetic form, and was approved in 2015 for the treatment of

advanced liposarcomas and leiomyosarcomas (BARONE et al., 2017; GORDON et al., 2016). This molecule exerts its antitumor functions through binding to the minor groove of the DNA double helix, which can causes structural and genomic machinery damages, but it also able to operate by a direct interaction of the compound with specific factors involved in DNA transcription and repair (LARSEN; GALMARINI; D'INCALCI, 2016).

Trabectedin attachment to DNA strands cause the inhibition of the DNA separation during the transcription process and the blockage of DNA repair systems, that leads to the generation of double-strand breaks in its structure. It also exerts the inhibition of active transcription by blocking and triggering the degradation of RNA polymerase II. Lastly, and perhaps the most important of all effects, trabectedin is able to displace oncogenic transcription factors from its target promoters, which leads to reverse of cell differentiation and other cancerous features (MINUZZO, 2005; SVEJSTRUP, 2007; D'INCALCI; GALMARINI, 2010; CHARYTONOWICZ et al., 2012; DI GIANDOMENICO et al., 2013). The effects of trabectedin on transcription factors and transcriptional activity is considered unique and opens up to new possibilities for anticancer drugs that bind to DNA.

Plitidepsin is another tunicate compound that was recently approved by Australian regulatory agency for clinical treatment of multiple myeloma in combination with dexamethasone for patients that relapse after three lines of treatment (www.ebs.tga.gov.au). The proposed mechanism of action for this compound is very unique with the inhibition of the elongation factor eEF1A2, resulting in the accumulation of misfolded toxic proteins in cancer cells, triggering apoptosis (for review see Jimenez et al., 2020).

Another very innovative concept is the use of antibody conjugates that enable the delivering of toxic molecules to specific tumor cells expressing the desired antigen. These systems included a monoclonal antibody that will guide the ADC to target cells, a potent cytotoxic compound and a linker that will be accountable for releasing the cytotoxic compounds inside target cells (Senter & Sievers, 2012; Jimenez et al., 2020). Adcetris was the first the first approved drug using this formulation in 2011 for the treatment of Hodgkin's lymphoma and systemic anaplastic large cell lymphoma, but the concept have been largely applied to different targets resulting in dozens of ADC in clinical trials and the recent approval of enfortumab vedotin-ejfv (PADCEV™) and Polatuzumamu vedotin (Polivy™) for the treatment of metastatic urothelial cancer and

non-Hodgkin lymphoma (and other hematological cancers), respectively (https://www.midwestern.edu/departments/marinepharmacology/clinical-pipeline.xml).

Reiterated by all these stimulants examples, marine organisms are recognized as prolific sources of molecules with unique mechanism of action. Even more promising, the obtention of natural products from marine microorganisms figures as an interesting alternative considering the issues of biotechnological versatility, material supply and the sustainability of the process (MANIVASAGAN et al., 2013) (FENICAL; JENSEN, 2006).

Salinosporamide A is a β-lactone isolated from the marine-restricted actinomycete bateria *Salinispora tropica*, and it in phase II of clinical tests for myelomas treatment (POTTS et al., 2011). Its mechanisms of action involve the linkage with the catalytic subunit 20S of the proteasome, which results in the inhibition of its functions and, consequently, inhibition of proteolysis regulation and cellular apoptosis activation (BORISSENKO; GROLL, 2007). It entered into clinical trials in record time - only 2 years after its discovery - and all tests were carried out with the natural product obtained from fermentations of *S. tropica* (FENICAL et al., 2008).

More recently, a new natural product isolated from the marine bacterium belonging to the genus *Serinicoccus*, the seriniquinone, demonstrated potent activity on several tumor cell lines, with particular selectivity for the Malme-3M melanoma cell line (TRZOSS et al., 2014). Using an immunoprecipitation strategy, researchers identified that seriniquinone targets to dermcidin protein in order to promote its cytotoxicity roles. In addition, high levels of dermcidin expression was found in the Malme-3M, which directly correlates the selectivity of seriniquinone for these cells.

Notably, natural products isolated from marine microorganisms appear as a rich source of new compounds with interesting activities. For more than 15 years, the research group conduct by Professor Leticia V. Costa Lotufo aims to investigate the pharmacological potential of marine species inhabiting the Brazilian coast. In this sense, bioactive molecules from marine invertebrates were isolated as lipid amino acids from the zoanthid *Palythoa grandiflora* (WILKE et al., 2009, 2010) and derivatives of 7-oxo-staurosporine from ascidia *Eudistoma vannamei* (JIMENEZ et al., 2012), among others (FERREIRA et al., 2011; TAKEARA et al., 2008). In 2009, studies on the biotechnological potential of marine bacteria were initiated, aiming the prospection of associated microbiota of marine invertebrates and sediments. Other bioactive

molecules were obtained from this source (ARTHAUD et al., 2012; GUIMARÃES et al., 2014; JIMENEZ et al., 2013; RODRIGUES et al., 2015; SAHM, 2014; SOUSA et al., 2012), highlighting the biotechnological richness of the huge microbial biodiversity present in Brazilian coast.

1.5 Prospection strategy - Bio-affinity chromatography

One of the biggest obstacles to the development of drugs from natural products is the long period required for their development (MARTINS et al., 2014). Despite this, the peculiar biological activity inserted in natural products continues to achieve this source as unique, not only for the development of new therapies but also as important tools in biological research (CARLSON, 2010). While new compounds with great potential are discovered in the marine environment, the development of methods that accelerate the connection between isolation and biological characterization are necessary, in order to reduce the time and costs inserted in the research.

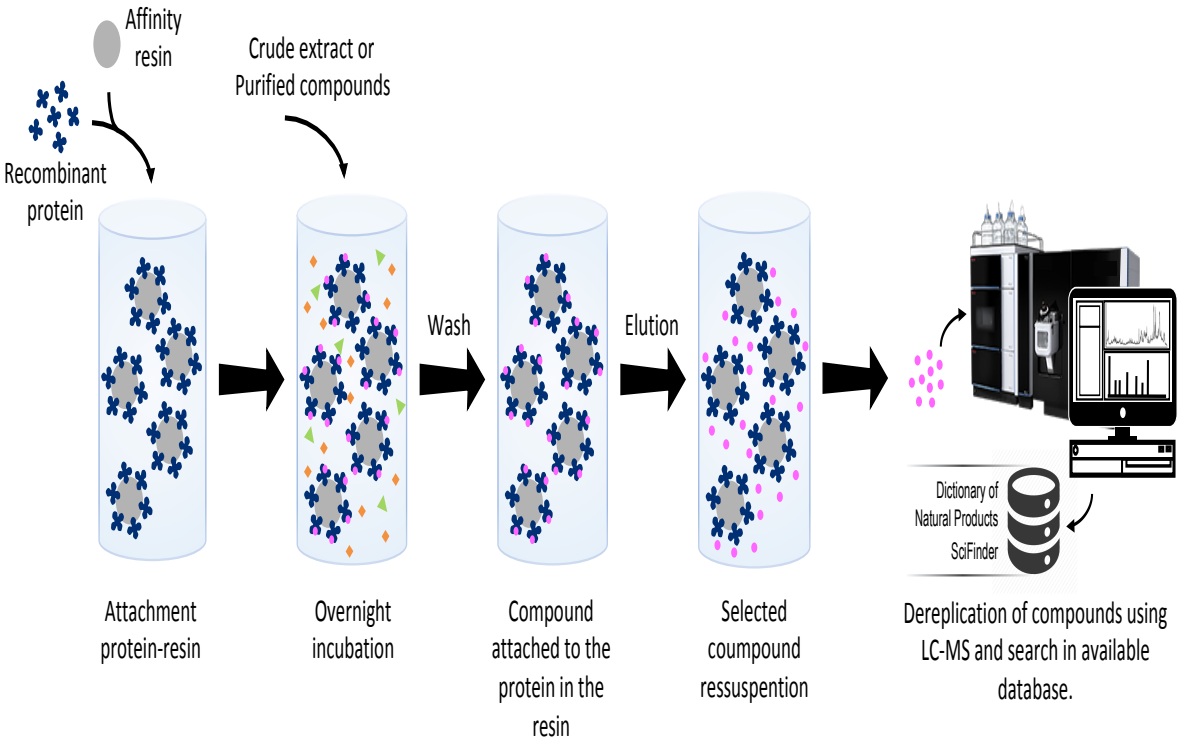
Traditionally, studies with natural products are carried out based on the observation of biological activity in organic extracts. From this point onwards, the active molecule is isolated and identified, guided by its activity. Posterior studies are then necessary to elucidate the mechanism of action responsible for the biological activity. Such studies are carried out through the evaluation in different biological tests, often without pointing to a specific target, making several steps necessary until the pathway by which the substance performs its functions is defined (HUGHES et al., 2011; AGARWAL et al., 2014).

In most recent years, the integration of DNA technologies into pharmaceutical research, opened up to a revolution of new strategies in the field of drug discovery and development. A molecular target-oriented approach quickly spread through the labs. In this strategy, which is called target-drug discovery (TDD), the molecular target important to the disease biology is firstly identified and its complementary DNA (cDNA) is used to express recombinant protein for purification, or to generate genetic engineering cell lines that overexpress the target. TDD assays relies on measuring the activity of recombinant molecular target of interest. Additional experimental studies that link the specific target to a disease model or condition is the bedrock for TDD strategies, a process that is denominated "target validation" (HUGHES et al., 2011; LEE; BERG, 2013). In general, phenotypic assays are frequently used as "next step"

experiments in TDD workflows, which shows that traditional and most-modern approaches are essential and complementary in drug discovery programs.

Aiming to reduce the number of steps of traditional procedures, and to optimize the isolation of substances that act on specific molecular targets, Lau and collaborators (2015) developed the method called functional (bioaffinity) chromatography, a reverse affinity procedure exemplified in figure 5 (LAU et al., 2015). The technique was built along the lines of a TDD prospecting approach, where a recombinant protein (biological target) is attached in an affinity resin following incubation a crude extract or a purified compound. Functioning as a fishery, where the fish is selectively attracted by the bait, this procedure aim the isolation of compounds with binding specificities with the molecular target. After separation, the identification of the compound or compounds of interest is done quickly by well-developed and highly sensitive chemical methods, such as nuclear magnetic resonance and mass spectrometry, enabling the detection of compounds in small quantities and precise structural determination.

Figure 5. Schematic exemplification of the reverse affinity technique (Bioaffinity chromatography) developed by Lau et al., (2015).



Firstly, the recombinant protein is attached to the affinity resin following by crude extract or purified compound overnight incubation. After washed procedures, the selected compounds are eluted from the affinity column next proceeding to chemical identification by LC-MS analysis. Scheme gently provided by Prof. Larissa Alves Guimarães.

8. FINAL CONSIDERATIONS

Although there are currently several anticancer therapeutic options in use, cancer remains one of the deadliest diseases in the world, which requires the development of new and more efficacious therapies. Melanoma is a type of cancer that has been increasing in its incidence rate. As it is a disease that mainly affects the elderly and has a carcinogenic relationship with UV radiation (UVR), the increase in the overall survival of the world population together with climate changes that enhance the incidence of UVR, new cases of melanoma tend to be more numerous and frequent in the next few years (BALATO et al., 2015). The relevance for developing new therapeutic strategies in the field of melanoma treatment is regarding to the lack of efficacy and cancer resistance to traditional and target-therapy (KOZAR et al., 2019).

The new biotechnological era has opened previously unimaginable doors for medical research. Advances in the development of efficient tools have enabled scientists to immerse themselves in understanding diseases and other biological processes. For example, The Cancer Genome Atlas (TCGA) program contributed significantly to uncover many drivers of tumorigenesis through progress in sequencing several cancer genomes (TOMCZAK; CZERWINKA; WIZNEROWICZ, 2015). The role played by transcription factors (TFs) in cancer has also reached great advances. TFs plays a pivotal role in regulating gene transcription and is found deregulated in many diseases, including cancer. It is estimated that around 20% of all oncogenes discovered so far are TFs (VAQUERIZAS et al., 2009; BRADNER; HNISZ; YOUNG, 2017).

The great interest in discovering approaches that modulate TFs functions in cancer is based on the fact that their deregulations occur specifically in cancer cells, which would result in the development of a specific therapeutic intervention with a reduction in adverse effects and patient-specific therapies (HAGENBUCHNER; AUSSERLECHNER, 2016). Even more, TFs are involved in diverse cellular process that ranges from replicative immortality to developing of resistance. The development of TFs target drugs figures as a great strategy in modulating many cancer properties at the same time, in contrast to the limited effects of various kinase inhibitors which are mainly regarding antiproliferative effect only (BUSHWELLER, 2019).

Nevertheless, for a long period, drug discovery researchers assumed that targeting transcription factors was impossible due its "undruggable" properties. In order to mediate TFs functions, interferences between protein-protein and protein-DNA interactions figured as the logical modulation of TFs. The challenges related to the "undruggable" features is due the fact that this factors present a large protein-protein interaction interfaces hardly occupied by a small molecule, lack of a deep protein pockets which difficult molecules accommodation, plus their intranuclear localization which in theory add further limits in accessing these targets (DALE et al., 2019; BUSHWELLER, 2019).

Yet, in cancer research, drugging an undruggable target is a key challenge that has been achieved before. It is important to highlight that many so-called undruggable targets were eventually developed as promising and successfully therapies. As examples, only a couple of decades ago tyrosine-kinases were considered difficult to target and nowadays inhibitors of this class of targets are widely used in personalized anti-cancer therapy (WU; NIELSEN; CLAUSEN, 2015). Moreover, it is now well proved that directly inhibition of TFs from BCL-2 family by small molecules are possible (ASHKENAZI et al., 2017; VAXMAN; SIDIQI; GERTZ, 2018), despite these factors possess all the "undruggable" features described to TFs above. Currently, many scientists believe that basic understanding of the biological and biochemical properties of TFs, using tools as X-ray crystallography and nuclear magnetic resonance, can make then druggable targets (DANG et al., 2017).

Actually, considerable progressions in modulating TFs functions for cancer therapy were achieved in the recent years (BHAGWAT; VAKOC, 2015; LAMBERT et al., 2018; BUSHWELLER, 2019). Most common approaches include targeting nuclear hormone receptor ligand binding domain; inhibition of TF-cofator protein-protein interactions; target chromatin remodeling/epigenetic readers protein in order to modulate expression of TF drivers; disrupting TF-DNA binding by directly binding TF's with small molecule or yet by compounds that binds to DNA preventing TFs recognition of its consensus sequence (HAGENBUCHNER; AUSSERLECHNER, 2016; BUSHWELLER, 2019).

Aiming at the development of new horizons for melanoma therapy, the present phD thesis starting point was the identification of marine natural compounds with binding affinity against the TF TBX2, previously known for their important oncogenic functions in melanomas (PRINCE et al., 2009; WANSLEBEN et al, 2013). Targeting

the TF DNA-binding domain directly by a small molecule, or other mimetic molecule, can changes its conformation or prevents its binding with DNA, thus cancelling its transcription functions. For these reasons, screening approaches use the TF DNA-binding domain as bait for identification of DNA-binding domain interacting compounds (HAGENBUCHNER; AUSSERLECHNER, 2016). Herein, we use a reverse affinity procedure where TBX2-DNA-binding domain recombinant protein was the bait. We found that the minor groove binder molecule, CA_5, has binding affinity to TBX2-DNA Binding domain and further investigations revealed that TBX2-driven melanoma cells are highly sensitive to these compound and up-regulation of TBX2 target genes, once repressed, were observed at low doses treatment.

Moreover, blockage of the interaction between protein/DNA-binding by compounds is an interesting approach for modulation of TFs functions, as mentioned above, and has proven to be efficient mechanism of action designated to DNA minor groove binders compounds. Identify compounds that compete with TF for their specific consensus sequence in the DNA, or molecules able to specific create conformational changes in the DNA that prevent the binding or recognition of TFs figures as good alternatives in inhibiting the activity plays by oncoTFs (HAGENBUCHNER; AUSSERLECHNER, 2016; BUSHWELLER, 2019). Notable, we also demonstrate that CA_5 have great binding affinity to the TBX2 DNA-target sequence, which configures this molecule as a potential modulator of the TBX2 functions in melanoma and other tumors that express this factor by either directly interaction with TBX2-DNA-binding domain or through blocking its specific binding sequence.

Still, the results found here represent the beginning of the investigation into the modulatory role that CA_5, or others minor groove binders, plays against TBX2. Several additional experiments still need to be performed in order to better clarify its mechanisms and further identifications of approaches for the development of a potential target-therapy. Moreover, the participation of TBX2 in the CA_5 cytotoxicity is currently being investigated in breast cancer and rhabdomyosarcomas cell lines as well, which will soon address new insights about the findings reported in the present study. In conclusion, we prove that marine natural products recovered from the Brazilian coast have the potential to produce interesting compounds with important biological activity. Also, we validate the reverse affinity procedure using the TBX2-DNA-binding domain as bait, which open this approach for high throughput screenings in order to identify more molecules with potential binding to TBX2 TF.

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