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A Review on Epigenetic Therapy and Implications in Cancer Tumor

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ABSTRACT
In this article, migration, invasion and pre-metastatic niche formation are all important events that occurin metastasis of a primary tumorand understanding all aspects of this process is essential toprevent cancer-related deaths, including melanoma. This review explored the significance, causesand treatments of melanoma, and summarized recent publications that highlight the role ofmelanoma-derived exosomes in the progression of the disease.
Keywords: Epigenetic; melanoma; tumor; cytotoxic.

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I. INTRODUCTION

Melanoma is derived from melanocytes, the pigment-forming cells of the skin, hair follicles,uvea, inner ear, nervous system and heart. Melanocytes originated from neural crest cells andcan produce melanin through a specialized membrane bound organelle known asa melanosome. Phenotypically, melanocytes are oval shaped, have dendritic arms and aretypically 7 micrometers in diameter. Dendritic arms found on melanocytes allow for cell-cell interactionwith keratinocytes, enabling the transfer of melanin-containing melanosomes from the melanocytesto the keratinocytes[1]. This transfer of melanin to keratinocytes determines the color of the skinand hair of an individual. Melanin functions as perinuclear protection against harmful ultraviolet(UV) radiation in keratinocytes, store ions, as a free radical scavenger, and couples' oxidation-reductionreactions. Interestingly, melanin has been demonstrated to have detrimental effects in vitro innormal human melanocytes, where it was shown to enhance single stranded DNA breaks, which couldbe explained by the formation of reactive oxygen species (ROS) during photo-oxidation of melanin inexperimental settings. Results from these studies suggest that the complex functions of melaninmay be cell type dependent.Normally, melanoma is detectable by the irregular shapes in pigmented lesions on the skin;however, there are exceptions that are difficult to differentiate. Several techniques have been developedover the years to better detect melanoma before it advances to metastasis, which is crucial in improving the diagnosis, prognosis and treatment of melanoma patients[2].

Molecular assays such as: Fluorescent In-Situ Hybridization (FISH), Comparative GenomeHybridization (CGH), Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR), NextGeneration Sequencing (NGS) and detection of exosomes have been shown to be of great value formelanoma detection. Melanoma tumor cells are characterized by certain chromosomal aberrations.The FISH technology uses various fluorescent probes to distinguish between benign and melanomatumors in unambiguous samples (87% and 95%, respectively), however its efficacy in ambiguoussamples is yet to be demonstrated.

Another method of melanoma diagnosis is CGH, this method involves extracting normal andtumor DNA and fluorescently labeling each DNA sample with fluorophores of different colors. The next step is to use the differentially labeled DNA probes and hybridize control DNA usingeither the metaphase chromosomes or DNA microarrays. The hybridization of the different coloredfluorescence probes to the control DNA will allow the determination of any chromosomal region gainor loss based on the colors. CGH allows for the better view of the genome allowing it to detectmultiple chromosomal anomalies compared to FISH, which can only detect limited loci.

qRT-PCR and NGS are technologies which may have diagnostic values, however they are still inrelatively early stages of development. qRT-PCR in a clinical setting will be used to determine the geneexpression patterns of tumor samples. The

successfullysuppresses NOTCH activation. However in phase 2 trials, only a modest responsiveness to GSIwas observed in metastatic melanoma patients[6].

When melanoma patients undergo chemotherapy, the tumors are frequently known to adapt andbecome resistant against monotherapies. In order to increase the efficacy of therapeutic treatments,combination therapies are commonly administered. In preclinical studies, BRAF inhibitor-resistantmutated BRAF melanoma cells were developed as an experimental model system to mimic BRAFinhibitor-resistant melanoma patients and to study the mechanisms of resistance. These cells weretreated with a combination of GSI and BRAF inhibitor, a reduction in cell growth and an increasein senescence were seen in these resistant cells, however, when they were weaned off GSI duringthe study, there was an increase in cell growth. These pre-clinical findings suggest that in a

combination therapy, which inhibits both MAPK and NOTCH pathways, may improve the efficacyof melanoma patients who develop BRAF inhibitor-resistance but both inhibitors must be present tosustain the anti-tumor progression responses.

Stage III melanoma patients along with surgery can be offered immunotherapy with interferon,which functions to enhance immune response, or the anti-cytotoxic T-lymphocyte-associated protein.For stage IV melanoma patients, they can undergo immunotherapy sessions in conjugation with othertargeted drug chemotherapy, and in some cases radiotherapy. Immunotherapies utilize the host'simmune system to elicit a tumor-specific immune response to combat cancer malignancies. Recentfocus for immunotherapies has been on dendritic cell (DC)-based cancer vaccinations. DCs are of greatinterest in cancer because of their ability to uptake, process and present antigens, which enables themto elicit an immune response. In the case of potential cancer vaccines, this immune response is againstthe cancer cells. Specifically, the use of dendritic derived-exosomes (DEXO), which are nanovesiclesreleased from DCs, and have shown promise, as they contain the machinery required to activate potentantigen-specific immune response. Damo et al. showed that DEXOs from DCs that were incubatedwith both a ligand for TLR-3 to stimulate the cytotoxic natural killer cells as well as the CD8+ cells,and melanoma antigens from necrotic B16F10 cells, the DEXOs were then injected into mice bearingB16F10 tumors and resulted in a significant reduction in growth of the tumors[7].

Another focus of immunotherapy is targeting immune checkpoints that function as tumor sample is given a score based on the gene expressionmeasurement, and it will be categorized as a benign lesion or malignant melanoma based on the givenscore. Furthermore, it could also allow physicians to distinguish between melanoma subtypes[3].

Additionally, the qRT-PCR is being utilized to better diagnose and design treatments for patients byway of predicting the metastatic risks in Stage I or II melanomas. NGS could be a great diagnostictool, due to its ability to sequence tumor DNA, not only in the coding regions that make up proteins,but also the regulatory regions that control the timing and levels of a given protein. These techniqueswill allow the clinicians to determine mutation-specific treatments; it will be interesting to see howthese approaches impact the treatment outcome of human cancers[4].

Treatment Methods

The treatment for primary melanoma patients is surgical removal of the tumor(s). Treatmentoptions for late-stage melanoma patients include targeted drug therapies with or without radiation orimmunotherapies. Many of the targeted therapies involve components of the MAPK/ERK pathway[5].

A well-known target is the mutated BRAF protein and small molecule inhibitors have been developedagainst it. The well-known BRAF inhibitors, vemurafenib/zelboraf (PLX4720/ PLX4032), havebeen shown to improve survival rates for many melanoma patients. There have been various inhibitors developed against other components of the MAPKpathway. In vivo, the MEK inhibitor, selumetinib, has shown to reduce melanoma xenograft tumorgrowth. Inhibitors of ERK have been shown to successfully inhibit the MAPK pathway inMEK-inhibitor resistant cells, since ERK is downstream of MEK. It has not been possible to developan inhibitor towards RAS. Difficulties in inhibition of RAS has led to the development of othertargets to indirectly inhibit the effector molecules of RAS by way of development of inhibitors of theRAF-ERK-MEK pathway such as farnesyltransferase, Rce1, lcmt1, PI3K-AKT-mTOR pathway andRalGEF-Ral pathway.

Malignant melanoma cells were shown to have higher levels of NOTCH signaling when comparedto normal melanocytes, indicating its role in melanoma pathogenesis. Under normal conditions, NOTCH signaling is required in the maintenance of Melanoblast and melanocyte stem cells, however,in mature melanocytes, low or undetectable levels of NOTCH expression are found. The gammasecretase inhibitor, GSI, was developed to target NOTCH signaling pathway and

cytoplasm of thecells they are released from, which naturally would suggest that the contents of melanoma-derivedexosomes are unique when compared to other tumor types, and even the normal cell counterpart.A study exploring this utilized 2D-PAGE analysis of both melanoma-derived exosomes and cell lysatesfrom which they originated, strikingly different proteomic profiles were observed. The exosomes

contained drastically less or were absent of lysosomal and mitochondrial proteins that were presentin the cell lysates. In contrast, the proteins that were enriched in the exosomes of SK-MEL-28 andMeWo melanoma cell lines included p120 catenin, radixin, and immunoglobulin superfamily member8 (PGRL). Exosomes

that were prominin-1 positive were isolated and analyzed, and were found to contain, along withCancers **2016**, 8, 110 9 of 18exosome-specific proteins, multiple pro-metastatic proteins, including CD44, MAPK4K, GTP-bindingproteins, ADAM10 and Annexin A2[8-9].

In clinical samples, exosomes were isolated from melanoma patients and found to containhigher concentrations of Melanoma Inhibitory Activity (MIA), a small protein secreted by malignantmelanoma cells and S100B, a calcium binding protein involved in cell cycle progression anddifferentiation, expressed by melanoma cells, when compared to healthy volunteers. Exosomesfrom liver perfusates of patients with metastatic uveal melanoma contained the protein melan-A.Not only are certain protein enriched in melanoma exosomes, studies show a multitude ofmiRNAs and their expression profiles are specific to these vesicles. A study by Ragusa et al. revealedthat exosomes derived from uveal melanoma patients contained a common miRNA in the exosomes,miR-146a, within the vitreous humor, as well as those circulating through the body. Another

miRNA in exosomes, miR-126b, was down-regulated only in patients with advanced melanomacompared to healthy donors. In another study, Felicetti et al. demonstrated that the metastaticability of melanoma cell lines was proportional to the amount of miR-222 within the exosomes.In a clinical setting, exosomes derived from the plasma of sporatic metastatic melanoma patientsdisplayed elevated levels of miR-17, -19a, -21, -126 and -149 compared to those with familial melanomaor healthy controls. Additionally, there was no differential expression of miRNAs seen in familialmelanoma patients and unaffected group. These results suggest that in familial melanoma, geneticpredisposition instead of miRNAs plays a critical component in the onset and progression of regulatorsof T-cell activation through receptor/ligand complexes. Another checkpoint target is the receptor/ligand,program death-1 (PD-1) and program death ligand-1 (PD-L1). Targeting this checkpoint pair wasshown to have anti-tumor activity in melanoma patients. PD-1 receptors interaction with itsligands, PD-L1 and PD-L2 in peripheral tissues, which induces a reduction in T-cell effector functionand enhances apoptosis. In metastatic melanoma, PD-L1 is upregulated along with tumorinvading lymphocytes and IFN-production; suggest a process by which melanoma tumors evadeimmune system attack. A phase I trial with the monoclonal anti-PD-1 antibody, nivolaumab,showed that 28% of the patients with advanced melanoma had a partial or complete response totreatment and out of those, 72% who received nivolaumab for more than a year were responsive totreatment that lasted for a year or more.

An alternative approach in conjunction with immunotherapies is natural compounds that havebeen explored as additional treatment options. Curcumin, a plant based chemical, has been shownto have anti-cancer effects including anti-angiogenic, pro-apoptotic and the ability to modify theimmune system. Curcumin, being a natural product, is generally less toxic than other syntheticdrugs, but the bioavailability in the body for curcumin is low. Several groups are developing noveldelivery systems such as nanoparticles, liposomes, micelles and phospholipid complex to increaseits bioavailability. Curcumin has been shown to mediate its anti-cancer effects by modulatingthe MST1, JNK, BIM-1, FOXO3, BCL-2, JAK-2/STAT-2, and BAX pathways, in in vitro models.

Specifically, in melanoma cells, curcumin was shown to induce apoptosis in a dose and time dependentmanner. In an advanced melanoma murine model, it was shown that treatment with amphiphiliccurcumin–based micelle led to remodeled tumor microenvironment and enhance vaccine efficacy.

A combination therapy using amphiphilic curcumin with vaccine therapy resulted in a downregulationof immunosuppressive factors as well as increased the efficacy of the vaccine treatment where therewas a 7-fold increase in INF-y and increase in cytotoxic T-cell response.

Exosomes and Cancer

There are numerous ways that have been proposed for melanoma-derived exosomes to participatein the survival, proliferation, and metastasis of melanoma.8.1. Unique Composition of Melanoma ExosomesThe composition of exosomes often reflects the contents of the membrane and

invasiveness of the MSCs. Xiao et al. also found that normal melanocytes could gainthe ability to invade when incubated with exosomes from melanoma cells. The pro-metastaticprotein, Met72, was detected in the highly metastatic clone of B16 melanoma cells (B16-10). B16-10exosomes express Met72 and can be taken up by the poorly metastatic clone of B16, B16-F1, whichthen begins to express Met72 and exhibit metastatic activity similar to B16-10 cells. Anotherexample of a pro-metastatic phenotype transferred to another cell via exosomes involves WNT5A[11-12].

In malignant melanoma cells WNT5A induces a calcium-dependent release of exosomes that containimmuno-modulatory and pro-angiogenic factors involved in metastasis that have the ability to induceimmune suppression and angiogenesis. It is hypothesized that melanoma exosomes induce therelease of vascular endothelial cell derived tumor necrosis factor alpha which causes thelymphatic endothelial cells to tolerate tumor growth within the nodes.

Interestingly, exosomes from other cell types have the ability, through exosome release, to increasepro-metastatic phenotypes. Exosomes released by adipocytes contain proteins involved in fatty acidoxidation (FAO), which are only found in these exosomes. They can be taken up by melanomacells induced elevated FAO levels and increased in migration and invasion. In another report,neural cell exosomes were shown to have the ability to affect the morphology and physiology ofmelanoma cells including activation of MAPK pathway within the cell, modulating melanogenesisand dendrite-like outgrowths of the cells, supporting the notion that exosomes from one cell type isable to influence the differentiation and cell signaling of another.

Melanoma cell-derived exosomes have been shown tomanipulate primary tumormicroenvironmentby: (1) supporting the epithelial-to-mesenchymal transition (EMT) of the cells in the melanocyticmicroenvironment, promoting metastasis, through autocrine/paracrine signaling activating the MAPKpathway. miRNAs involved in this transition, let-7i, mir191 and let-7a, were shown to be presentin circulating exosomes from stage 1 melanoma patients but not in exosomes from non-melanomapatients; (2) Affecting the differentiation of immune cells by enhancing the maturation of dendriticcells and T-cell proliferation; (3) Activating macrophages when treated with melanoma-derived

exosomes and exhibit a different cytokine and chemokine profile than when exposed by other activatorssuch as LPS or IL-4; and (4) increasing migration of endothelial cells and inducing thedisease, miRNAs could be used as a prognostic and diagnostic tools in patients with non-familialmetastatic melanoma[10-11].

Not only do exosomes contain special proteins and miRNA, the lipid bilayer that encases theexosome macromolecules are unique to melanoma exosomes. Melanoma exosomes are composedof lipid bilayers that contain a high concentration of sphingomyelin and high levels of tetracaineproteins, which is hypothesized to be the determinant in the release of the exosomes.

Many studies have shown that exosomes from melanoma cells and of plasma from melanomapatients contain certain proteins and miRNAs. This has tremendous potential in diagnosis andprognosis of melanoma. For example, patients with an increased concentration of MIA in theircirculating exosomes correlated with a shorter median survival rate. Additionally, detectionof metastasis has great potential using these vesicles; a group found that the profiling of exosomalmiRNA from metastasis in the liver showed differences when compared to the primary tumor.Understanding the unique composition of melanoma exosomes has the potential in detecting lesionsand influencing the course of treatment of melanoma patients and continues to contribute to ourunderstanding of the progression of the disease.The unique composition of exosomes released from melanoma cells may contribute to their abilityto manipulate other cells. Melanoma cell derived exosomes have been shown to induce tumorigenesisor induce pro-metastatic behaviors in other melanoma cells, as well as normal cells. These exosomesseem to contain different determinants from the cells they originate from, which may be involved inpromoting metastatic behavior. For example, miR-222 has been shown to play a tumorigenic role inmelanoma, by its ability to induce the PI3K/AKT pathway and this miRNA can be transferred fromthe exosomes to the recipient cell and cause subsequent induction of the PI3K/AKT pathway.

Interestingly, Nieto and colleagues identified several unique proteins only found in exosomes fromhighly metastatic melanoma cell lines. These proteins are known to be involved in cell motility,angiogenesis and immune responses, suggesting the transfer of pro-migratory proteins from the highlymetastatic exosomes to the less aggressive ones.

Indeed, the characteristics of a cell line that exhibits a metastatic phenotype has the ability totransfer proteins to recipient cells that gain some of those characteristics. For example, exposure ofmesenchymal stem cells (MSCs) to exosomes from Prominin-1-positive melanoma cells resulted in aCancers **2016**, 8, 110 10 of 18increased

immune cells have been shown to interact with melanomaexosomes; RNA from either melanoma cells or Lewis lung carcinoma cell-derived exosomes are takenup by lung epithelial cells and result in activation of Toll-like receptor-3 (TLR3) in these cells andcauses the infiltration of neutrophils. This infiltration promotes pre-metastatic niche (PMN) formationin the lung. TLR3-deficient mice do not form lung metastases and have a reduction in PMN formationdue to a decrease in neutrophil infiltration.

Melanoma exosomes have also been implicated in the promotion of angiogenesis by regulatingendothelial cells from a distanceand manipulate cytokine expression profiles to establish animmunosuppressive environment.Commonly, when patients are undergoing various treatment regiments, the tumor initiallyshrinks, then seems to spontaneously develop resistance and begins to resume growth, regardlessof continuation of treatment. There have been recent studies suggesting the involvement ofmelanoma-derived exosomes in treatment resistance. Melanoma cells also have the ability to createan acidic microenvironment. This reduction in pH is a mechanism of inducing resistance for thesecells from cisplatin treatment. When the cells are co-treated with proton pump inhibitors and cisplatin,exosome release is reduced, in addition to a higher pH, and an increased amount of uptake of cytotoxiccisplatin by the cells. Melanoma cells have also been shown to accumulate chemotherapeuticagents within vesicular compartments and release them in exosomes, as shown by Chen et al. withcisplatin treatment. Melanosome release is enhanced in the presence of cisplatin where they areexploited for cisplatin removal from the cell.

II. CONCLUSIONS

In this review, we discuss on epigenetic therapy and implications in cancer tumor. Cancer is the second leading cause of death in the United States, and about 6% ofthe estimated cancer diagnoses this year will be melanoma cases. Melanomas are derived fromtransformation of the pigment producing cells of the skin, melanocytes. Early-stage melanoma isusually curable by surgical resection, but late stage or subsequent secondary metastatic tumorsare treated with some success with chemotherapies, radiation and/or immunotherapies. Mostcancer patients die from metastatic disease, which is especially the case in melanoma.

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In addition to the ability of melanoma-derived exosomes to affect the local cellular environment,these exosomes also have been shown to travel throughout the body and accumulate in distant organs.Peinado et al. demonstrated the distribution and metastatic potential of B16-derived melanomaexosomes in the lung and was supported by results from Morishita and colleagues wherethey demonstrated the distribution of inoculated radio-labelled B16BL6 murine melanoma-derivedexosomes throughout the body, and found that after a very short half-life in circulation, theseradio-labelled exosomes accumulated in the lung, spleen and liver. Similar observations were madeearlier with the gLuc-LA-coupled B16BL6-exosomes[15].

The premetastatic niche (PMN) is the site of possible secondary metastasis. This microenvironmentis made up of multiple different cell types, including fibroblasts, infiltrating immune cells, endothelialcells, and other cells that comprise the blood and lymph vessels. These and the extracellular matrixmust create a supportive microenvironment for the arrival, growth and establishment of a secondarytumor from the circulating tumor cell destined to arrive there.Melanoma exosomes may play an important role in the formation of the PMN. They have beenshown to induce vascular leakiness at pre-metastatic sites, an event that is important in the formationof the niche. Exosomes injected into xenograft tumor bearing mice showed changes in mRNA profilingof the lungs, mainly in those that are involved in various steps in pre-metastatic nicheformation. BoneCancers **2016**, 8, 110 11 of 18marrow progenitor cells also accumulated in pre-metastatic niches. These exosomes probablyinduce molecular signals that help melanoma cells prepare sentinal lymph nodes for metastasis, recruitother critical molecules, ECM deposition and vascular proliferation within the nodes[16].

Tumor-derived exosomes are hypothesized to also be involved in manipulating interactionsbetween the origin tumor cells, and the surrounding tissue stroma to promote malignancy. Specifically,those exosomes have the ability to interact with immune cells, which then help manipulate themicroenvironment to be conducive for metastatic growth. For example, human melanoma andcolorectal carcinoma-derived microvesicles have been shown to promote the differentiation ofmonocytes to myeloid-derived suppressor cells that support the growth of the tumor and the ability toescape immune surveillance. Other

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