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REVIEW: PROTEINS c-Myc AND Myc-nick AS POTENTIAL TARGETS FOR THE MELIGNANT MELANOMA TREATMENT

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Abstract

Malignant melanoma is the most aggressive and life-threatening skin cancer with increasing incidences over the past decades. Despite accounting for only 4 % of all skin cancers, melanoma confers 80 % of skin cancer induced death. The underlying cause of melanoma progression and metastasis is poorly understood.

Myc is a very strong proto-oncogene and it is very upregulated in many types of cancers. c-Myc protein is a transcription factor that activates expression of many genes. It drives cell proliferation, plays a very important role in regulating cell growth, apoptosis and differentiation. High c-Myc expression is associated with tumor metastasis and poor prognosis in human melanoma.

Full-length c-Myc is converted into Myc-nick by calcium-dependent on cytosolic proteases that are members of the calpain family. In connection with the key role of the cytoplasmic protein Myc-nick in the autophagy activativation, increasing the resistance to chemotherapy and overall survival of tumor cells, it can be a target for treatment tumors under

the conditions of c-Myc overexpression. That fact that the expression of Myc-nick increases the survival of cells after UV radiation can indicate the key role of the Myc-nick in tumorogenesis of melanoma cells.

Key words: **c-Myc; Myc-nick; autophagy; apoptosis; melanoma.**

Introduction

Malignant melanoma is the most aggressive and life-threatening skin cancer with increasing incidences over the past decades. This disease is prevalent in faired-skin populations, and is easy to detect due to its abnormal colour, size and pigmentation. Despite accounting for only 4 % of all skin cancers, melanoma confers 80 % of skin cancer induced death. Although, melanoma has high recovery rates if detected early, it has a high tendency of metastasizing, dropping the 5-year survival rate less than 5 %. The underlying cause of melanoma progression and metastasis is poorly understood, hence limiting the effectiveness of current treatments and contributing to increased cases of recurrence or refractory melanoma. Thus, it is vital that future anticancer agents can combat both local and metastatic melanoma [1].

Many vital biological processes are tightly regulated by complex signalling networks and signal transduction occurs when an external stimuli (e.g. stress, UV) initiate changes in a cell upon binding of ligand to surface receptor. This causes conformational changes to the intracellular signalling molecules, which in turn elicit cellular responses. Disruption of these pathways or intracellular communication can result in various diseases, including cancer [2]. Thus, signalling molecules have become potential targets to halt cancer progression by inducing apoptosis and/or inhibiting tumour metastasis and angiogenesis.

During tumour metastasis, high oxygen delivery and consumption requires activation of hypoxia-inducible factors (HIFs), which induce transcription of growth-related genes such as vascular endothelial growth factor (VEGF). The activation of VEGF can stimulate tumour angiogenesis and increase the oxygen delivery [3]. These changes in hypoxic cancer cells allow them to acquire invasive and metastatic properties as well as to develop resistance to chemotherapy [4].

Presently antibodies that inhibit endothelial growth factor receptor (EGFR) (cetuximab, panitumab), tyrosine kinase inhibitors (gefitinib, erlotinib) and substances that blocking the relationship of EGFR with ATP (imatinib) are used as targeted therapy for cancer [5]. But unfortunately in the case of protein mutations involved in the implementation of KRAS / BRAF / ERK / MAPK and PI3K / Akt / mTOR signaling pathways using of this

targeted therapy has not any therapeutic effects [6]. In this connection the search for target proteins that are associated with these signaling pathways and present at the cascade beginning of signaling events leading to malignant cell regeneration is of great importance. Inhibition of the activity of these proteins could block the progression of the tumor process and metastasis, regardless of the mutations of proteins located on the overlying sections of the signal pathways. One of such representatives are the proteins of the Myc family. The Myc transcription factors are considered as promising therapeutic targets because their increased expression is observed in many types of human tumors such as myeloma, lymphoma, carcinoma, neuroblastoma. An increased level of the L-Myc gene mRNA is detected in ovarian cancer while N-Myc plays a key role in the development of retino- and neuroblastomas tumors common happened in children [7]. High c-Myc expression also is associated with tumor metastasis and poor prognosis in human melanoma [8].

Aim: Determination of the c-Myc and Myc-nick role in the pathogenesis of malignant melanoma by the analysis of literary data with a retrospective at 5 years.

Results and discussion

The protein c-Myc encoded by this gene is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. Myc protein is a transcription factor that activates expression of many genes through binding enhancer box sequences (E-boxes) and recruiting histone acetyltransferases (HATs). It can also act as a transcriptional repressor. By binding Miz-1 transcription factor and displacing the p300 co-activator, it inhibits expression of Miz-1 target genes. In addition, Myc has a direct role in the control of DNA replication. Myc is activated upon various mitogenic signals such as serum stimulation or b Wnt, Shh and EGF (via the MAPK/ERK pathway). By modifying the expression of its target genes Myc activation results in numerous biological effects. The first to be discovered was its capability to drive cell proliferation, but it also plays a very important role in regulating cell growth (upregulates ribosomal RNA and proteins), apoptosis (downregulates Bcl-2), differentiation, and stem cell self-renewal. Myc is a very strong proto-oncogene and it is very often found to be upregulated in many types of cancers. Myc overexpression stimulates gene amplification, presumably through DNA over-replication [9].

It was noted that all clones expressing the transfected c-Myc gene show reduced class I HLA mRNA and beta 2-microglobulin mRNA expression in melanoma cells. These results show that the class I HLA expression is modulated by the level of c-Myc expression, thus opening up the possibility that high expression of this oncogene influences the interaction of

melanoma cells with the immune system [10]. But the antigenrepresentative role of c-Myc into the melanoma cancerogenasis is still unknown.

Recently much attention has been paid to the study of the c-Myc role in regulating the proliferation of tumor cells and the activation of angiogenesis through EGFR signaling pathway. It is known that mTOR stabilizes the Myc protein [11]. Recent studies show that the protein synthesis is not only enhanced by the transcriptional activity of Myc but also by activating the mTOR-dependent phosphorylation of 4EBP1 [12]. In anothers study it was showed that Myc protein regulation is controlled by mTOR through the AMBRA/PP2 complex protein [13]. Also it was repoted that intestinal tumor growth and angiogenesis are activated through upregulation of VEGF expression mediated by the MEK/ERK/c-Myc pathway [14]. In another source it was demonstrated that EGFRvIII induces Angptl4 expression through the ERK/c-Myc pathway and promotes tumor angiogenesis in malignant gliomas [15]. But using EGFR ingibitors and antibodies can not always give positive result in cancer treatment because blocking EGFR can lead to the autophagy induction what may be a cause of chemoresistance [16, 17]. The role of c-Myc into the autophagy activation at this case must be investigated. It is necessary to determine whether it can act as a target to prevent chemoresistance after blocking EGFR not only for malignant melanoma treatment but another tumors.

JNK1 and JNK2 play redundant functions in Myc-induced B cell lymphoma formation [18]. JNK is induced by nutrient deprivation and activates Bcl-2 phosphorylation as a gatekeeper in autophagy activation. It has been repotted some role of JNK 1-mediated by Bcl-2 phosphorylation in autophagy and apoptosis regulation. At the first time of starvation Bcl-2/ Beclin 1 complex is subdivided and it leads to autophagy activation. With prolonged starvation higher levels of Bcl-2 phosphorylation leads to disruption of the complex between Bcl-2/Bax promoting apoptosis at a time point when autophagy can no longer keep cells alive. It was marked by increasing level of caspase 3 in 16 hours of starvation [19].

Also it was repoted that MAPK regulates the activation of several transcription factors and can activate c-Myc by phosphorylation [20]. But the influence of JNK and MAPK signaling pathways into the c-Myc expression and their role into the regulation of autophagy and apoptosis processes in the case with malignant melanoma unfotunatly are unknown.

Taking into account the negative effect of hyperexpression of c-Myc in various tumors the creature of medicines aimed at inhibiting Myc family oncogenes is an important direction in antitumor therapy. Today there are a number of strategies aimed at blocking Myc function in tumor cells. Some of them are aimed at inhibiting the expression of Myc genes at the level

of transcription or translation, while others are blocking the protein-protein interactions necessary for the function of Myc as a transcription factor for example the formation of the Myc / Max heterodimer [21-23].

Of particular interest is the presence of Myc-nick protein in the cytoplasm of tumor cells with the overexpression of the Myc gene. Cytoplasmatic staining of Myc is not an artifact of fixation. Myc-nick is a transcriptionally inactive form of Myc that is localized in the cytoplasm. This N-terminal truncation of Myc, named Myc-nick, performs an active role in the cytoplasm that is independent on Myc's transcriptional activity. Full-length Myc is converted into Myc-nick by calcium-dependent on cytosolic proteases that are members of the calpain family. Unlike proteasomal degradation leading to total protein destruction, calpain-mediated proteolysis cleaves Myc to generate Myc-nick, which comprises the N-terminal region of Myc from residues. Aberrant calpain levels and activity have been correlated with tumor development and metastasis. Furthermore, several immunohistochemical studies have described a cytoplasmic signal for Myc in tumors of diverse origins. Myc-nick is expressed in tumor cell lines and tissues. Myc-nick was determined in lymphoma, thymic tumors, prostate cancer, neroblastomas, medulloblastomas and colon carcinoma. Myc-nick can cause different dramatic morphological changes of cells: long protrusions in cellular membrane, occasional forms of intercellular contacts. Myc-nick augments cancer cell motility by inducing fascin expression and filopodia formation [23]. Perhaps such changes in the cell membrane are associated with the possibility of Myc-nick to activate proteins glycosylation of the cytoplasmic membrane of tumor cells which promotes their adhesion and metastasis. It is known that Myc-nick promotes migration and survival of cells in response to chemotherapeutic agents or withdrawal of glucose [24]. Expression of Myc-nick in tumor samples raises the possibility that this form of Myc may contribute to a fundamental aspects of tumor cells biology [25, 26]. The information of regulative function of Myc-nick in melanoma cells line is unknown. Moreover the fact that UV treatment promoted the conversion of Myc into Myc-nick within 5 min of irradiation in Rat1 fibroblasts and the expression of Myc-nick increases the survival of these cells after UV radiation [19] can indicate the key role of the Myc-nick in tumorogenesis of melanoma cells.

It is known that the concentration of Myc-nick depends on cell cultivating conditions. Hypoxia and nutrient deprivation lead to cytoplasmic accumulation of Myc and conversion into Myc-nick [19]. It is interesting the fact that elevated levels of full-length Myc promote programmed cell death under starvation conditions [19], but the opposing data were also filled, where it was indicated that Myc-nick protects cells against apoptosis under stress

conditions [27]. This may indicate the role of Myc-nick into the autophagy activation in tumor cells under stress conditions. In this case, apoptosis must precede autophagy at the initial stages of carcinogenesis, when the expression of c-Myc begins to increase. This is also confirmed by the fact that Myc-nick expression causes adramatic increasing of LC3B-II and LC3A-II (autophagy markers) in DLD1 cells without affecting the levels of the autophagy-inducing factors ATG3, ATG5, ATG7, and ATG. There is a statement that Myc-nick promotes autophagy that is at least in part responsible for the increased resistance to stress-induced apoptosis [25].

From another side Myc-nick-expressing cells sensitive to apoptosis caused by glucose and glutamine deprivation and increases cleaved caspase 3 [27]. Thus it can be concluded that apoptosis precedes autophagy under conditions of nutrient deprivation in order to support the autophagy. There are publications confirming this statement. The p53 protein wich is responsible for apoptosis can transactivate a number of factors (DRAM, IGF-BP3, PTEN, TSC2, AMPK, etc.) that block the anabolic activity of mTOR and thereby activate autophagy. Inhibition of the p53 activity leads to decreasing in autophagy signaling and promotes tumorogenesis. It is found that the action of p53 depends on its cellular localization. With cytoplasmic localization it inhibits autophagy. And the induction of p53 accompanied by its movement into the nucleus activates autophagy in many ways [28].

The role of Myc-nick in the regulation of apoptosis into the cells can be explained also by the fact that overexpression of Myc-nick protein increases activity of transacetylases what leads to acetylation of tubulin and another proteins, including p53. Not so long ago it has been shown that the acetylation of lysines in the C-terminal domain of the p53 protein results in the ability to bind to certain regions of the DNA activating apoptosis. Recently, it has been studied that the negatively charged domain of the SET protein binds to the positively charged C-terminal domain of the p53 protein and inhibits p53 activity. In the state of cellular stress, the lysine residues of the C-terminal domain are acetylated and the SET complex is not formed, and p53 remains active [29, 30]. Thus the Myc-nick helps not only protect cells from apoptosis, but also can activate it through acetylation of p53. However the switching mechanisms and factors affecting their implementation remain unknown. Perhaps acetylation of p53 is also one way of activating autophagy processes aimed at increasing overall cell survival.

Conclusion and prospects

Considering the fact that malignant melanoma is one of the more aggressive tumors and in 80 % cases leads to a lethal outcome, the search for target markers for early diagnosis and targets for the treatment of this oncopathology is of great importance. Taking into account that malignant melanoma is characterized by high expression of c-Myc and Myc-nick respectively and also that these proteins perform an ambiguous role in the regulation of apoptosis and autophagy by the example of other oncopathologies, their role in the carcinogenesis of melanoma should be studied in more detail. Because exactly c-Myc and Myc-nick can be one of the proteins that may make chemoresistance in the treatment of malignant melanoma by activating autophagy aimed at increasing the overall survival of tumor cells. In this case Myc and Myc-nick can be targets for the treatment of melanoma and tumors of different locations. The main way to reduce the formation of cytoplasm Myc-nick is the inhibition of cytosolic proteases that are members of the calpain family in tumors with c-Myc overexpression. This targeted therapy would increase the activity of apoptosis and decrease the ability to autophagy of tumor cells, which would contribute to a decrease in cell survival and resistance to chemotherapy. It simplies a perspective in this scientific direction.

Since in recent years a great importance is given to the development of antitumor immunotherapy, it would also be interesting to study the antigen-presenting role of c-Myc and to determine the autophagy role in the regulation of immune surveillance in carcinogenesis of malignant melanoma.

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Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All information is publicly available and data regarding this particular patient can be obtained upon request from corresponding senior author.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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