Molecular and Genetic Mechanisms in Melanoma

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ABSTRACT

Recent studies have indicated an increasing incidence of melanoma worldwide. Although UV signature mutations are found rarely in melanoma cells, there is some evidence that intense intermittent exposure to sunlight can induce melanocyte tumorigenesis, and this is also observed after UV irradiation in some animals. The purpose of this paper is to review some of the most important mechanisms involved in the pathogenesis of this tumor. Genetic studies showed that familiar melanoma is linked to the mutation or deletion of the suppressor gene CDKN2A, and perhaps to CDK4. Studies showed that BRAF mutation is frequent in primary and metastatic melanoma cells but also in naevocytic nevi. This mutation activates the RAF/MEK pathway. Exposure to UV radiation induces immunosuppression. Recent investigations showed that chemokines, angiogenesis, metalloproteinases can play a role in the mechanism of metastasis. In spite of these advances the initiating events are still not completely understood. In conclusion, the pathogenesis of melanoma is very complex because numerous genetic and epigenetic factors are implicated in its development and progression, but some of the showed mechanisms can be targets for new therapies.

Key words: suppressor genes, oncogenes, pathogenesis, therapy

Introduction

Melanoma as a «fatal black tumor with metastasis» had been already described in the Corpus Hippocraticus¹. Recent epidemiologic studies have found an increasing incidence of melanoma at an alarming rate in several parts of the world²-⁴. The tumor develops from melanocytes in the skin, but can arise also in the mucous membranes, uveal tract of eyes, and leptomeninges. The factors implicated in the fast rise in the incidence of melanoma are incompletely understood. In part this can be explained by the change in life style, changes in clothing, vacational and weekend sunbathing, longevity, stratospheric ozone depletion⁶-⁷. The melanoma etiology is multifactorial: both genetic factors (familial melanoma, xeroderma pigmentosum, fair skin) and environmental factors such as sunlight (especially the UV radiation) contribute to its inception and evolution⁸. The role of other, non sunlight risk factors, like exposure to ionizing radiation, exposure to chemicals used in some occupations have been also related to melanoma, but possible interactions with genetic factors and UV irradiation is to take in to account⁹-¹¹. Immunity can also be involved.

In recent years, there has been much concern for humans regarding solar or artificial UVA in the induction of melanoma¹². Although UVB signature mutations C-T and CC-TT transition¹³ are found rarely in melanoma cells, there is some evidence that intense intermittent exposure to sunlight can induce melanocytes tumorigenesis, and this is also observed after UV irradiation in some animals¹³-¹⁵. The purpose of this paper is to review the knowledge about the mechanism of initiation and progression of melanoma.

Hallmarks of Malignant Tumors

During the last decades the understanding of the pathogenesis of tumors has increased considerably but some aspects are still not fully understood. Cancer cells develop from «normal» cells through the accumulation of genetic alterations, which finally leads to the malignant phenotype.
Hanahan and Weinberg have reviewed the most important alteration in a cell leading to malignant transformation and proliferation:

- self-sufficiency in growth signals (growth factors, their receptor up-regulation, or growth signaling pathways like ras – raf, MAP cascade);
- insensitivity to antigrowth signals (pRb lost by mutation of its gene or sequestrated by viral oncoproteins);
- acquisition of resistance toward apoptosis (defect in activation of FAS death signaling circuit or a mutation in p53 in response to damage of DNA via signal for proapoptotic Bax);
- acquisition of limitless replicative potential (up-regulation of telomeres with maintenance of telomere length, circumvention of cellular senescence);
- sustained angiogenesis;
- tissue invasion and metastasis.

The stepwise progression is possible if there is a genome instability. Below we will underscore these aspects in melanoma.

**UV Effects**

It is well established that outdoor or indoor exposure to UV radiation is associated with the development of skin cancer: basal cell carcinoma and squamous cell carcinoma. The tumor appears in sun-exposed areas (head, neck, upper limbs), mostly in people with fair skin, frequently on photoaged skin and with actinic keratoses. Acute and chronic UV exposure to UVB (290–320 nm) causes direct DNA damage. DNA absorbs UVB energy by double bound on adjacent pyrimidine bases, thymine (T) and or cytosine (C) on the same strand, forming a four-member cyclobutane ring and pyrimidine 6,4 pyrimidine (T) and or cytosine (C) on the same strand, forming a four-member cyclobutane ring and pyrimidine 6,4 pyrimidine photoproducts. There is C to T transition or CC to TT double base mutation. These changes on p53 are fingerprints or signature mutations of UVB photocarcinogenesis. Experiments in hairless mice and in xiphophorus hybrid fish confirmed the mutagenic effects of UVB.

For melanoma it is not so simple to link it to sunlight. Epidemiological studies showed that melanoma, unlike other skin cancers, is associated with an intense intermittent exposure to sunshine (trunk and legs), or use of tanning lamps, especially in childhood. A recent study showed a significant risk for melanoma among individuals who used tanning beds in comparison with those who never used them. For long time UVB was believed to be the «bad UVR» and UVA the safe. Experiments in animals demonstrated that the relevant carcinogen radiation, in melanoma, may be UVA, which causes oxidative damage in the cells. Solar UVA (320–400 nm) makes up approximately 90–95% of UV rays that reach the earth’s surface. Their energy is small; however, they penetrate deeper into the skin. A growing body of evidence indicates that UVA, even if absorbed weakly by DNA, causes skin aging, and generates a variety of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, peroxinitrite, singlet oxygen i.e. an oxidative stress. The epidermis contains antioxidant enzymes such as catalase, glutathione peroxidase but intense UV can overwhelm their effects. ROS indirectly damages DNA, forming purine oxidative (mainly guanine) photo-products such as 8-oxo-7,8-dihydro-2’deoxy-guanosine, G-T transitions and strand breaks. These alterations were demonstrated in experiments on animals and human fibroblasts. Moreover, ROS can facilitate metastases damaging the endothelial cells.

UVA inducing matrix metalloproteinases, via AP-1 and NF-xB, increases the aggressivity of the skin cancer. So, UVA radiation, apart of inflammation, and immunosuppression can also induce photocarcinogenesis.

**Gene Mutations**

The majority of melanomas arises «de novo» from melanocytes, which function is the synthesis of melanin, its storage, and transfer to keratinocytes. Some melanomas arise from nevi, especially from dysplastic and giant congenital nevi. There are four clinic-histological types of melanoma: the most common is the superficial spreading melanoma, then nodular melanoma, lentigo maligna melanoma and acral lentiginous melanoma. So it seems unlikely that only one mechanism or few genes can have a role in the origin and progression of melanoma cells.

The pathogenesis of melanoma is complex because numerous genetic, epigenetic factors (hyper- or hypomethylation of DNA, acetylation), and environmental factors are implicated in its development and progression. The mutation, deletion, amplification, translocation or methylation of suppressor genes or activation of proto-oncogenes are of paramount importance in the mutagenesis. The mutations of oncogenes are dominant: change in one allele has a determinate effect and can stimulate the growth or proliferation of the cell. Clark et al. proposed that the development of melanoma (melanomagenesis) is commonly stepwise: nevus, dysplasia, melanoma with radial growth phase, vertical growth which is associated with metastasis. For the clinician and pathologist it is important weather melanoma is in the radial metaphase or in the vertical growth phase, when metastases are common.

In 1820, Norris already wrote that black tumors and moles may be hereditary. Numerous epidemiological and genetic studies showed the familial melanoma, which represents 8–12% of all melanomas, is linked to the mutation or deletion of the suppressor gene CDKN2A, and occasionally with mutation of the protooncogene CDK4, CDK6. In familial melanoma patients there is commonly an aberrant regulation of the cell cycle by cyclins with cyclin dependent kinases: they carry a germ line mutation in the locus cyclin dependent kinase inhibitor 2A gene (CDKN 2A) on the chromosome 9p21. It encodes two suppressor proteins p16 (inhibitor kinase 4A-INK4A) and p14 or Arf (alternative reading frame). The reading frame encodes p16 whose function is to inhibit the complexing ciklin D/CDK4 and CDK6 i.e. enzymes that...
phosphorilate the retinoblastoma protein (pRB) and so drive the cell division cycle. pRB phosphorilation consequently releases E2F which among others, drives the G1 in S phase of the cell cycle. Recently, Wang et al. found that protein E2F1 has also a suppressor function through p53 or p73. Mutation of CDKN2A is found in 10–30% of the cases in familial melanoma. The mutation or silencing of p16 has been found also in sporadic cases of melanoma, some with C-T transitions, and also together with mutation of the oncogene N RAS.

Patients with CDKN2A mutation can present multiple melanosomas. Mutation or loss of CDKN2 2A occurs rarely also in actinic keratoses, skin carcinomas, lung carcinoma, breast carcinoma, and other tumors.

The CDKN2A β reading frame encodes p14 (ARF) whose function is to sequester the Mdm2 protein, a negative regulator of p53 mediating its degradation in the proteasome. Loss of ARF decreases p53 activity and apoptosis of mutated cells. Recent experiments demonstrated in mice models that p14 is a suppressor and can induce even senescence in melanocytes activated by oncogenes. So, mutation of CDKN2A results in loss of pRB, p53 control of cell regulation and evasion of senescence.

The development of melanoma is linked also to the skin color. Melanoma occurs in whites tenfold more frequently than in blacks, while it is rare in albinos. This correlation depends mostly on the amount of melanin. So, fair skinned people (skin types I and II) with blond or red hair are more predisposed to melanoma. This is associated with the function of the melanocortin 1-receptor gene (MC1R), located on chromosome 16q24. The gene is associated with the function of the melanocortin 1-receptor (MC1R), which encodes the transmembrane G protein receptor.

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The gene CDK4 (chromosome 12 p14) mutation, whose protein is a kinase, has also been found in some melanoma families, and this lead to cycle progression. Experiments in transgenic mice expressing a mutant CDK4 have shown that the inactivation of retinoblastoma/p16 pathway, leads to the development of melanoma. Melanoma can develop also in patients suffering from hereditary retinoblastoma i.e., with germ line mutation of Rb1 gene (13 q14), and in patients with mutations or amplifications of CDK4.

Patients with xeroderma pigmentosum, in which nucleotide excision repair (NER) is defective, are characterized by a high sensitivity to sunlight, with sunburns, and freckling. The UVR induced DNA damage is not recognized or incorrectly repaired, it remains mutated and this leads to an early development of precancerous lesions, nonmelanoma skin cancer, and melanoma. Interestingly, patients with xeroderma pigmentosum, who develop melanoma have frequently mutated p53, a rarely mutated gene in sporadic melanoma. Moreover, transgenic mice without these genes have increased photosensitivity and are prone to develop skin tumors.

Another DNA mutation found in melanoma, is the protoonco gene BRAF (chromosome 7q34) which encodes a cytoplasmatic serine/threonine kinase in the ras pathway. BRAF mutation is present in approximately 60% of the cases, and is the most frequently mutated gene in this tumor, especially on the non sun exposed areas, or better on the intermittent exposed areas (trunk, legs). BRAF activates by phosphorylation other protein kinases: MEK-ERK (which enter the nucleus)-MAP cascade. The mutation hits the exon 15 with a substitution of valine with glutamic acid at position 600 (val 600 glu). So, the substitution of only one aminoacid leads to mutation. Studies showed that BRAF mutations are frequent in primary and metastatic melanoma cells, but also in nevocytic nevi. This mutation activates the RAF/ MEK pathway. Because of BRAF frequent mutation in nevi, it influences the proliferation than tumorigenesis, and probably is an early event that must be accompanied by other gene alterations. A recent international study by Curtin et al. on 126 cases of melanoma has demonstrated that in mucosal and acral melanosomas there is commonly a loss of the CDKN2A locus, while on the trunk is more frequent BRAF mutation.

Among the many genes found to be mutated in melanoma, there is also the suppressor gene phosphatase and tensin (PTEN), which is located on chromosome 10q23 and encodes a phosphatase that regulates the extracellular growth signals through the lipid phospho-tidylinositolphosphatase (PIP3). PTEN is inactivated by mutation, reduced expression or deletion in 25–50% of non-familial melanoma. The mutation activates through PIP3 AKT and so promotes the cycle progression and inhibits apoptosis. So, PTEN seems to be a regulator of apoptosis, cell proliferation, and cell adhesion. There may be a later mutation, and recently it was found an epigenetic silencing of the gene by promoter inactivation. Furthermore, PTEN is important for the integrity of the chromosomes, as its mutation is associated with frequent chromosome translocation. Mutation of BRAF and PTEN occurs frequently together.

Mutations in ras genes (1p13) are present in about 20% of human melanomas and are found also in nevi. Commonly the isoformal N-Ras is present. This mutation is observed frequently in melanoma from sun-exposed areas, and in congenital nevi. Studies demonstrated that N-RAS and BRAF mutations are not present in the same melanoma.
In human malignant tumors, the most frequently mutated gene is the suppressor p53 located on chromosome 17p13. Its protein product is a transcriptional factor that activates the transcription of numerous genes. p53 has a pivotal role in preserving genetic integrity: It is implicated in DNA repair mechanisms, apoptosis (through Bax), and cell cycle control through its effector p21. Investigations in melanomas showed that p53 mutations are rather rarely detected (about 10%), mostly in a later phase, and even less in nevi. However, the tumors grew rapidly if the mice were exposed to UVR. Probably this was linked to a loss of immunity, because the effect was transferable to other animals with T lymphocytes. These observations lead to the hypothesis that UVR suppressed the immune system both locally and systemically. So UVR inducing immunosuppression interferes with immunological mechanism of tumor immunosurveillance. First it was believed that only UVB could induce DNA damage, and so trigger immune suppression. Recent works demonstrated that also UVA can produce the same effect by altering the antigen presentation through depletion of Langerhans cells from the epidermis, induction of regulatory T cells (treg-formerly suppressor T Cell), production of IL-10, TNF α. The result is down-regulation of the cellular immune response such as induction and elicitation of contact hypersensitivity, and also an inhibition of NK cells activity, while the humoral part of the immune response is not altered.

Immunosuppression induced by UV can arise from damage of DNA, presence of the urocanic acid in the stratum corneum, which after exposure to UVR isomerizes in the cis configuration that has an immunosuppressive action or UVR leads to the formation of free radicals and membrane lipid peroxidation causing immune suppression. UV irradiation of keratinocytes releases neuropeptides and αMSH, which has immunomodulatory effects. Finally UVR can induce immunosuppression through transformation of provitamin in vitamin D. Despite the long story of immunotherapy in advanced stages of melanoma, the results are scantily.

Recent investigations showed that growth factors, chemokines, angiogenesis (in which VEGF, PFG, and IL-8 are implicated), matrix metalloproteinases, could play a role in the mechanism of melanoma progression and metastasis. In spite of these advances the initiating events are still not completely understood.

Conclusion

This brief review of the genetic and immunologic mechanisms of melanomagenesis underscores the relevance of UV irradiation, mutation or deletion of some oncogenes and suppressor genes, immune suppression, and telomerase activity in the initiation and progression of the tumor. It seems that of paramount importance are mutations that lead to clonal expansion (RAS, BRAF), changes that disconnect senescence (activation of telomerase), and evasion of apoptosis. The knowledge of such mechanism will be of significance for a new molecular classification of melanoma, has importance in better prognosing the outcome, and permits the development of new molecular target therapy for tumors.
GENETSKI I MOLEKULARNI MEHANIZMI U NASTANKU MELANOMA

SAŽETAK

Novija istraživanja ukazuju na porast broja melanoma širom svijeta. Unatoč tome što se signaturne mutacije rijetko opažaju u melanomskim stanicama, postoje dokazi da intenzivno i intermitetno izlaganje UV zrakama može prouzročiti malignu transformaciju melanocita, na što upućuju eksperimenti na životinjama izloženim UV zrakama. U ovom smo se radu osvrnuli na važnije mehanizme uključene u patogenezu ovog tumora. Genetske studije obiteljskog melanoma ukazale su na udruženost s mutacijama ili delecijom supresornog gena CDKN 2A, a vjerojatno i gena CDK4. Isto tako utvrđena je češća pojava mutacije BRAF-a u primarnim i metastatskim melanomima, a također u pigmentnim madežima. Ta mutacija aktivira RAS-MEK signalni put. Izlaganje UV zračenju uzrokuje i imunosupresiju. Novija istraživanja su pokazala da kemokini, angiogeneza i metaloproteinaze mogu imati ulogu u nastanku metastaza melanoma. Unatoč svim postignućima početni događaji još nisu potpuno razjašnjeni. U zaključku treba istaknuti da je patogeneza melanoma vrlo složena, te da u razvoju melanoma značajnu ulogu imaju genetski, epigenetski i čimbenici okoliša. Neki od spomenutih mehanizama mogu biti cilj novih terapija.