

Molecular genetics of non-melanoma skin cancer^{*}

Genética molecular aplicada ao câncer cutâneo não melanoma^{}*

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Abstract: Non-melanoma skin cancers are the most common malignant neoplasms in humans. About 95% of all non-melanoma skin cancers are represented by basal cell carcinoma and squamous cell carcinoma. Their prevalences are still increasing worldwide, representing an important public health problem. The genetic alterations underlying basal cell carcinoma and squamous cell carcinoma development are only partly understood. Much interest lies in determining the genetic basis of non-melanoma skin cancers, to explain their distinctive phenotypes, biological behaviors and metastatic potential. We present here a molecular genetic update, focusing on the most frequent genes and genomic instability involved in the development and progression of non-melanoma skin cancers.

Keywords: Carcinoma, basal cell; Carcinoma, squamous cell; Chromosomal instability; Loss of heterozygosity; Microsatellite repeats; Skin neoplasms; Skin neoplasms/genetics

Resumo: Os cânceres cutâneos não melanoma são as neoplasias malignas mais comuns em humanos. O carcinoma basocelular e o carcinoma espinocelular representam cerca de 95% dos cânceres cutâneos não melanoma, o que os torna um crescente problema para a saúde pública mundial devido a suas prevalências cada vez maiores. As alterações genéticas que ocorrem no desenvolvimento dessas malignidades cutâneas são apenas parcialmente compreendidas, havendo muito interesse no conhecimento e determinação das bases genéticas dos cânceres cutâneos não melanoma que expliquem seus fenótipos, comportamentos biológicos e potenciais metastáticos distintos. Apresenta-se uma revisão atualizada da genética molecular aplicada aos cânceres cutâneos não melanoma, em especial ao carcinoma basocelular e carcinoma espinocelular, enfatizando os mais frequentes genes e os principais mecanismos de instabilidade genômica envolvidos no desenvolvimento dessas malignidades cutâneas.

Palavras-chave: Carcinoma basocelular; Carcinoma de células escamosas; Instabilidade cromossômica; Neoplasias cutâneas; Neoplasias cutâneas/genética; Perda de heterozigidade; Repetições de microssatélites

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INTRODUCTION

Generally speaking, cancer is a disease caused by genetic mutations which confer the cells some special characteristics, like an unlimited proliferation capacity, loss of response to growth inhibition factors, evasion of apoptosis (programmed cell death), ability to invade other tissues (metastases), and production of new blood vessels (angiogenesis). Thus, most of the non-inherited malignant skin tumors result from mutations caused by carcinogens which somehow cause damage to the DNA and provide advantages which favor cell growth and the invasion of other tissues.

Non-melanoma skin cancer (NMSC) is the type of cancer with the highest incidence in Brazil in both sexes, with an estimate for 2006 of 116,000 new cases.¹ Since they are malignant neoplasms with low lethality and good prognosis, it is likely that the records are underrated due to underdiagnosis, to their indolent characteristic and to the onset of an adequate and timely treatment. Therefore, the estimates of NMSC incidence rates and expected numbers of new cases should be considered as minimum estimates.¹

Approximately 95% of NMSC are represented by squamous cell carcinoma (SCC) and by basal cell carcinoma (BCC), the latter being the most frequent skin malignancy, as it represents about 75% of NMSC in the western world.² In the US, BCC is the most diagnosed type of cancer, with an estimate of roughly one million cases per year.³

The risk factors that contribute to the development of NMSC are well known and include mainly ethnicity, age, gender, chronic exposure to chemical and physical mutagens, besides genetic factors.⁴ Excessive exposure to ultraviolet (UV) radiation, especially type B (UVB), has been associated to an increased risk of developing skin cancers including BCC and SCC,² because it can cause gene mutations in the deoxyribonucleic acid (DNA) of keratinocytes, and failure in the repair of these gene alterations can lead to a disorderly cell growth and to tumor formation.⁵ Moreover, UV radiation has a major effect on the immune system of the skin, inducing a local immunosuppression status that impairs the rejection of the newly formed tumor.⁶

Basal cell carcinoma

Malignant transformation of a cell in the basal layer of the epidermis or the appendages gives rise to basal cell carcinoma (BCC), a tumor that presents as its main characteristics indolence and slow growth, being locally destructive and rarely producing metastases.

The non-inherited (sporadic) forms of BCC

represent the absolute majority of diagnosed cases, while inherited forms are rarer and take part in a number of syndromes, such as the basal cell nevus syndrome (BCNS).

BCC displays differences in its biological behavior which can be explained by the presence of intrinsic factors of the tumor itself, such as tumor growth pattern, recurrence and metastasis potential, histological pattern and genetic factors.⁷ Extrinsic factors, such as site of origin, chosen therapy, and the immunologic status of the neoplasm patient, are also important.

From the histological viewpoint, variable growth patterns are observed which confer the BCC differences in its biological behavior. The classification based on growth patterns has greater biological significance and considers the existence of the nodular, superficial, infiltrative, sclerodermiform, micronodular and mixed-pattern types,⁸ which is useful in defining high- and low-risk histological subtypes of BCC.

High-risk BCCs are characterized by an increased probability of subclinical extension, incomplete excision, aggressive invasion or local recurrence, and include the superficial, infiltrative or sclerodermiform and the micronodular types.

Squamous cell carcinoma

Squamous cell carcinoma (SCC), or epidermoid carcinoma, represents about 20% of skin malignancies. It consists of an atypical proliferation of squamous cells, of invasive nature, which can produce metastases. As a rule, primary skin SCCs originate in sun-exposed regions, and there is no doubt that chronic and cumulative exposure to UV radiation, especially to UVB, is the primary cause of skin carcinogenesis.⁹⁻¹¹ While sporadic BCCs develop "*de novo*", SCCs can also arise from precancerous lesions, such as actinic keratoses (AK), actinic cheilitis, oral leukoplasias, and chronic radiodermatitis. However, other extrinsic factors can play an important causal role, including other forms of radiation, chemicals such as hydrocarbons and arsenic, tobacco, burns, human papillomavirus (HPV) infection, chronic ulcers, among others.¹²

The biological behavior of SCCs resembles that of epidermoid carcinomas originated from other squamous epithelia, and their histological differentiation is based mainly on the intensity of keratinization. The well-differentiated forms usually exhibit small keratin foci inside tumor lobes (corneal pearls), parakeratosis, besides little mitotic activity and minimal pleomorphism. On the other hand, poorly differen-

tiated SCCs demonstrate pronounced pleomorphism and little or no ability to produce keratin. In cases of intense anaplasia, an immunohistochemical examination can help identifying the epithelial origin of the tumor.¹²

Basic concepts in molecular genetics

Before proceeding, it is of fundamental importance to expose some basic concepts which will help understanding the molecular genetics of NMSC.

Gene organization and structure

The word “genetics” derives from the Greek root *gen*, which means “to become”. It was used for the first time in 1906, by Bateson, to designate the study of heredity and the variability of living beings.¹³

Genetics developed since the mid-19th century with Mendel’s studies on heredity, further with the clarification of the DNA structure by Watson and Crick in 1957)¹⁴ up to the complexity of the molecular biology of our days, when a few genomes are already entirely known, such as, for example, the human genome.

The term “genome” designates the complete set of sequences in the genetic material of an organism. It includes the sequence of each chromosome and also of any DNA contained in the organelles.¹⁴ The current stage of knowledge of bioinformatics allows the identification of protein coding sequences based on well-defined, but not exclusive, parameters. According to these criteria, it is estimated that the human genome possesses a little over 30000 genes, which correspond to approximately 10% of genome.¹⁵

In molecular terms, a gene is a sequence of DNA needed for the synthesis of a ribonucleic acid

(RNA) molecule, which can lead to the synthesis of a functional protein, thus following what was named the “central dogma of molecular biology” (Figure 1A).

Genes are preceded by the so-called promoter regions which are responsible for the regulation of their activation (expression). They are composed of coding regions (exons) intercalated with regions which, in principle, are non-coding (introns) (Figure 1B). During the processing of the pre-mRNA, the introns are removed, and the exons, linked to each other precisely and in the same order as they were in the respective gene, by a process named splicing. The exons contain the nucleotide sequence necessary for the amino acid synthesis of a protein during translation in the ribosomes. Although most genes encode proteins, some of them encode different types of RNA, such as transfer RNA (tRNA) and ribosomal RNA (rRNA), among other types.¹⁶

Proto-oncogenes and tumor suppressor genes

There are two main classes of genes which can undergo mutations and contribute to the genesis of cancer: the oncogenes and the tumor suppressor genes.³

Genes which act in the sense of stimulating cell division that may lead to uncontrolled growth are called proto-oncogenes. These genes are active during the embryonic phase and inactive in the adult cells. Many proto-oncogenes are growth-signaling molecules which, when mutated (oncogenes), stay perpetually “on”, generating the amplification of the cell growth signals and overcoming the normal controls imposed by cell homeostasis.

By and large, oncogenes are genetically dominant, so that a mutation in one copy of a proto-onco-

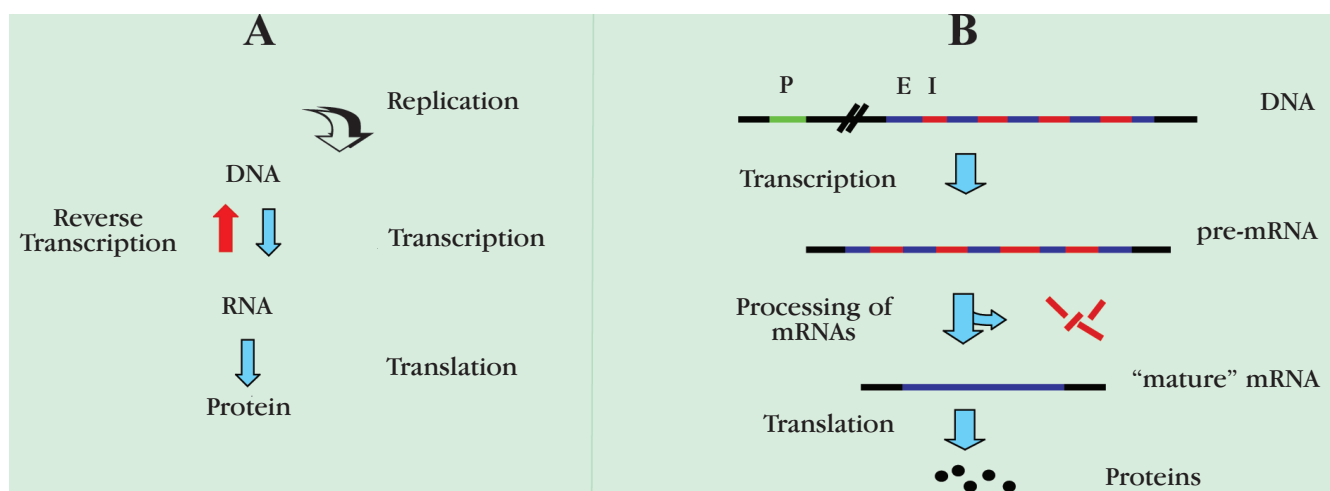


FIGURE 1: (A) Scheme representing the “central dogma of Molecular Biology”; (B) Representation of the structure of a gene and processing a transcript RNA. Promoting region in green (P), exons in blue (E), introns in red (I)

gene is sufficient to produce the phenotype. Examples of proto-oncogenes are the genes N-RAS, H-RAS, K-RAS and c-MYC.^{3,17}

Whereas the proto-oncogenes promote cell growth, there is a class of genes, named tumor suppressor genes, that acts by inhibiting the cell division cycle. As opposed to the oncogenes, which can act in carcinogenesis through the alteration of a single allele producing gain of function (dominant effect), tumor suppressor genes need a loss of function of both alleles to occur, thus characterizing a recessive effect. Tumor suppressor genes act by negatively regulating the cell growth signals, allowing DNA repair to occur. They act in the control and arrest of the cell cycle, being also able to trigger the programmed cell death process (apoptosis).¹⁸

It is a well established fact that an accumulation of gene alterations can lead to the development of cancer. This phenomenon has been extensively investigated by several authors, mainly in colorectal cancer, the model of which is considered ideal for understanding the carcinogenesis process, due to the progression from premalignancy to malignancy. The majority of cancers arise from a mutational inactivation of suppressor genes or from the activation of oncogenes.¹⁹

Chart 1, adapted from Fearon and Vogelstein,²⁰ explains the sequence of genetic mutations that occurs in the evolution toward colorectal cancer involving genes APC, K-RAS, DCC and TP53.²⁰ The model presented suggests the steps involved in the malignant transformation of colon cells, exemplifying how oncogenes and suppressor genes act during this process.

In the same manner as knowledge of the processes that culminate with the development of colorectal cancer can contribute to improved clinical

applications, whether diagnostic or prognostic, the study of the processes involved in BCC and SCC tumorigenesis may attain the same objectives in the future.

The involvement of some oncogenes and tumor suppressor genes in the development of cancers and related syndromes, such as melanoma,^{21,22} CBC,²³ neuroblastoma,²⁴ cancer of the pancreas,²⁵ of the ovary,^{26,27} non polyposis colon cancer,²⁸ BCNS,²⁹ retinoblastoma,³⁰ Li-Fraumeni syndrome,³¹ multiple sporadic cancers,³² among others, are represented in charts 2 and 3.

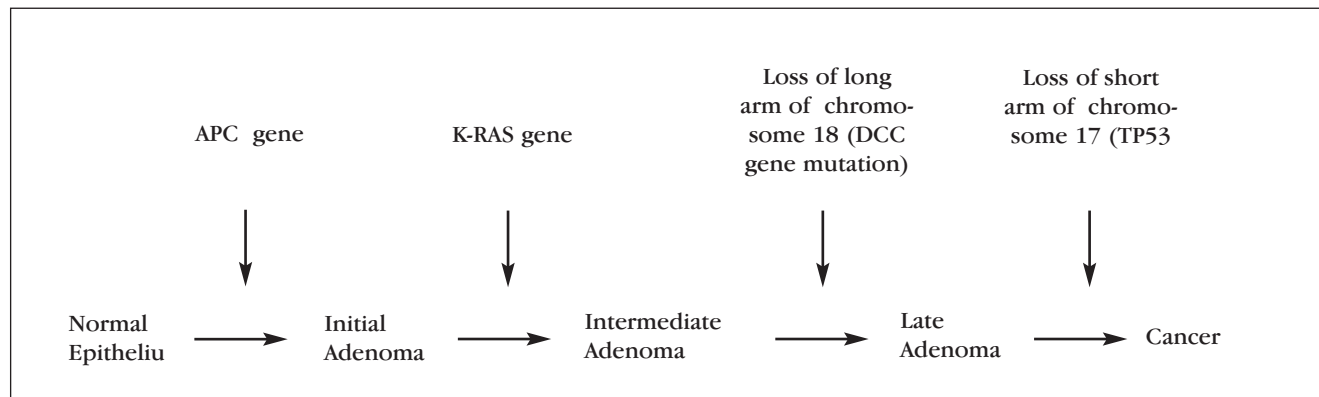
Protein p53 and the cell cycle inhibitors

When damages to the DNA occur, biochemical mechanisms are triggered in the cells and are capable of repairing such lesions. For this process to occur, the cell has to stop the cell cycle in order not to perpetuate the mutation, to activate the repair system and, if failures occur in these processes, to promote cell death. Evolution was able to give all these functions to a certain gene, TP53.

Due to its importance, the protein p53 expressed by this gene has commonly been referred to as the “guardian of the genome”. Mutations in gene TP53 are among the most frequent alterations found in cancer.³³ When damages to the DNA occur, biochemical mechanisms are triggered in the cells and are capable of repairing such lesions. For this process to occur, the cell has to stop the cell cycle in order not to perpetuate the mutation, to activate the repair system and, if failures occur in these processes, to promote cell death. Evolution was able to give all these functions to a certain gene, TP53.

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CHART 1: Model showing the genetic mutations that occur in progression from colorectal adenoma to carcinoma*



*modified from Fearon et al.²⁰

CHART 2: Examples of oncogenes and types of tumors associated to their gain of function

Gene	Types of associated tumors
MYC	Burkitt lymphoma, Lung and breast cancer
BRAF	Melanoma
GLI1	Basal cell carcinoma
K-RAS	Melanoma
N-MYC	Neuroblastoma
ABL	Chronic myeloid leukemia

TP53 are among the most frequent alterations found in cancer.³⁴

A non-functioning protein p53 due to a mutation is incapable of inhibiting cell division in the presence of damages, allowing the proliferation of cells with errors. The accumulation of such cells can lead to the activation of oncogenes or to the loss of function of the tumor suppressor genes. Loss of function of p53 can also inhibit apoptosis and thus increase the survival of altered cells.⁸

Mutations in gene TP53, typically induced by UV (change of nucleotides from C♦T and CC♦TT), can be found in up to 60% of BCCs.³² So far, the causal role of the frequency of these mutations in the development of BCC has not been demonstrated. Patients with Li-Fraumeni syndrome (inherited TP53 mutations) are susceptible to an increased incidence of internal tumors; however, higher incidence of skin cancers in these patients has not been described. Some authors suggested that TP53 mutations in BCC

CHART 3: Examples of suppressor genes and types of tumors associated to their loss of function

Gene	Types of associated tumors
APC	Sporadic and familial colorectal cancer Gastric and pancreatic cancer
BRCA1/BRCA2	Sporadic and familial breast cancer Cancer of the ovary
MSH2	Hereditary non polyposis colon cancer
PTCH	Sporadic basal cell carcinoma and BCNS [*]
RB	Sporadic and familial retinoblastoma
TP53	Multiple sporadic tumors Li-Fraumeni syndrome

^{*} Basal cell nevus syndrome

could be secondary, occurring after the initiation of the tumor.³⁵

Recent studies on gene TP53 have demonstrated mutations of the "UV signature" type, i.e., predominant conversions of C(C)♦T(T). Thirty-three percent of patients with BCC of Korean origin presented mutations in p53,³⁶ which attained 50% in Caucasian patients.³⁷ These data suggest that so far unknown ethnic differences may play a role in BCC carcinogenesis, even if diverse sun exposure patterns may also account for the differences observed.³⁵

Preliminary studies suggested that at least 90% of SCCs and 50% of BCCs presented mutations in this gene.³⁸ Currently, it is known that about 50% of all skin cancers are mutated, a frequency increased to as much as 90% when malignancy arises in patients with the recessive genetic disease named xeroderma pigmentosum (XP).³⁹ Most mutations occurring in TP53 in SCC are of the "UV signature" type, i.e., indicating mutations induced by UV radiation.^{40,41}

Recently it has been proposed that mutational hotspots in codon 177 of gene TP53 are specific for BCC, whereas mutations in codon 278 seem to be specific for skin SCC.⁴²

Despite this evidence, understanding the specific mutations of gene TP53 is still a goal to be reached, for better understanding of the mechanisms involved in the development of skin cancers.

Early and primary mutations in gene TP53 are found in epidermal keratinocytes^{9,11} and can be detected by immunohistochemistry.

It has been suggested that approximately 10% of precancerous lesions induced by sun exposure, such as for example AK, can undergo transformation into SCC;⁴³ therefore, it is not surprising that mutations of gene TP53, particularly of the UV type, are frequently found in AK.

Bowen's disease (BD), also known as carcinoma *in situ*, represents a pre-invasive stage of skin SCC. Both AK and BD can be immunopositive for p53,⁴⁴ and this precursor status is also suggested by molecular and cytogenetic studies.⁴⁵⁻⁴⁷

Other proteins like p16^{INK4}, which are cell cycle inhibitors, also play an important role in epithelial transformation. In normal cells, this cyclin-dependent kinase inhibitor (CDK) specifically impairs progression toward phase G1 of the cell cycle, blocking CDK-4, which thus does not phosphorylate the retinoblastoma protein (rb).⁴⁸ Locus INK4a also encodes another tumor suppressor that is structurally and functionally independent,⁴⁹ p14^{ARF}. The p14^{ARF} activates the p53 pathway in response to signals from oncogenes such as c-MYC or RAS, by binding to the negative regulator of p53 (Mdm2), thus preventing the degradation of p53

and, as a consequence, inducing cell cycle arrest or apoptosis.

There are discussions about the fact that mutations in p16^{INK4} can be late events in the development of skin cancers⁵⁰ and that the loss of heterozygosity (LOH), including the loss of whole parts of 9p, is frequently observed in SCCs.^{51,52} However, two recent immunohistochemical studies which evaluated the expression of p16^{INK4} in AK, BD and SCC of the skin showed controversial results.^{53,54} Mortier et al. suggested that progression from AK to SCC is correlated to the deletion of 9p21, locus of gene CDKN2A, which encodes p16.⁵⁵

Role of the patched gene in the development of BCC

Two patched (PTCH) genes, located at 9q22.3 (PTCH 1) and 1p32 (PTCH 2), were correlated to BCC. The identification of mutations in these genes, especially PTCH 1, as the cause of BCNS has contributed to a better understanding of the origin of BCC. BCNS is an autosomal disease characterized by the development of multiple BCCs, among other

anomalies.⁵⁶ Mutations in PTCH and in associated genes have also been demonstrated in sporadic BCCs.⁵⁷

Patched is a receptor for ligands of the hedgehog (hh) family of proteins and is located in the plasmatic membrane of cells.⁵⁸ These proteins are important in the modeling of human tissues during the embryonic period. The “hh” to “ptch” ligation induces the release and activation of another protein located in the membrane, named smoothed, expressed by gene SMO. Activation of smoothed, in turn, activates the transcription factor Gli1, which induces the transcription of several genes⁵⁶ (Figure 2).

However, even if there is a lot of evidence correlating the deregulation of the *ptch* pathway to the genesis of BCC, there is still little knowledge about how this cell defect exerts its tumorigenic effect.

All cells of patients with BCNS present a mutation in one of the PTCH alleles. A second mutation or loss of heterozygosity in this region is observed in tumors from these patients. Similar alterations have been identified in many sporadic BCCs, and the mutations are those typically induced by UV radiation. An increase in the expression of PTCH mRNA suggests that mutations in this gene are causal in the tumor development and not simply a marker of the UV effect. Transgenic animal models also suggest that mutations in this pathway contribute significantly to the formation of BCCs.⁵⁹

Deregulation of the *ptch* pathway does not explain the different histological subtypes of BCC, nor its different behaviors *in vivo*. As tumor development is a multi-step process, it is expected that alterations of other genes are involved in BCC carcinogenesis.

Therefore, it becomes evident that the action of both TP53 and PTCH contributes to prevent the development of BCC, which characterizes them as tumor suppressor genes.

If we attentively observe figure 2, we can see that two genes (SMO and GLI1) which take part in the *ptch* pathway are proto-oncogenes. If a gain-of-function mutation occurs in one or both of them, the BCC tumorigenesis process can be triggered, for their activation leads to the transcription of genes which favor cell growth.

However, review of the literature so far shows one single article on the possible role of GLI1 mutations in BCC, although no mutation was found in the cases studied.⁶⁰ The same authors found mutations in gene SMO in only 10% of cases.

In other oncogenes, like BRAF,⁶¹ N-RAS, K-RAS and c-MYC,⁴² no or few mutations were found in

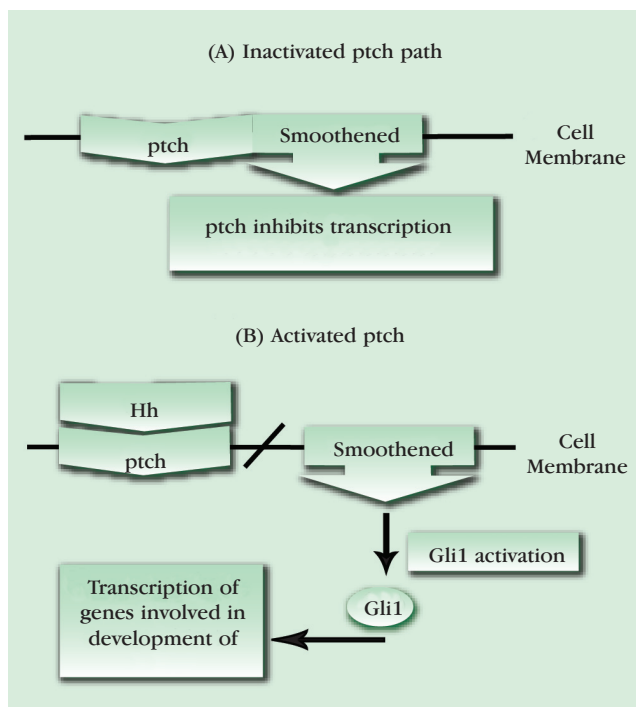


FIGURE 2: Signaling mechanisms of patched (ptch)/smoothened (smo): (A) in the absence of hedgehog (hh), smo is “kidnapped” by ptch, a membrane receptor of the basal cells of the epidermis, thus intracellular signaling will not occur; (B) in the presence of hh, it bonds to ptch, which, in turn, leads to the release of smo. The release of smo activates Gli1, which acts in the transcription of several genes involved in the tumor progression of BCC. Mutations in ptch eventually result in the release of smo without hh being present. (Modified from Dicker et al.⁵⁶)

BCCs, suggesting that these genes may not be involved in the development of this neoplasia.

Participation of extragenic DNA in cancer

While 10% of human genome is represented by genes, 90% correspond to DNA sequences with different repeat levels. Depending on their location in the genome, the repeated sequences have an influence on the organization of the chromatin and on the regulation of gene expression. Additionally, they play a fundamental role in rearrangements between sequences which lead to the development of countless diseases and to the evolution of genomes and species. Alterations in these sequences lead to the genomic instability observed particularly in different types of cancer. Among the repeated sequences which are important in the study of skin cancers, microsatellites stand out,⁶² which are short repeated sequences of one to six nucleotides, located at well-defined sites along the genome of eukaryotes, including humans. Many microsatellites are located next to important oncogenes and suppressor genes, which has led to the great interest in these regions as genetic markers.^{63,64}

DNA of tumor cells usually presents alterations in the number of repeated units in one or more microsatellites as compared to the same microsatellites from DNA samples of a normal tissue of the same individual.⁶⁵ Such tumor cells can also present defective "fingerprints" in their DNA as compared to other, normal tissues of the organism.⁶⁶ These "fingerprints" are sequences located between repeats distributed all along the genome, detected by the RAPD (random amplification of polymorphic DNA) technique and which characterize genetic instability. Alterations that culminate with an increase or decrease

in the number of repeats are defined as microsatellite instability (MSI) (Figure 3), whereas the complete loss of a microsatellite is known as loss of heterozygosity (LOH). The occurrence of MSI in tumor alleles is a direct indicator of the fact that failures occurred during cell replication and were not duly corrected, thus producing changes in the number of repeats in these alleles.

Alterations in 12 out of 18 microsatellites were found in BCC, two of which were located next to the suppressor genes MSH2 and TP53.⁶⁷ High frequencies of alterations are also seen in microsatellites which are close to the patched gene.⁶⁸ Recently, 60% of alterations in microsatellite D6S251 were demonstrated in region 6q14, in inherited and sporadic BCCs.⁶⁹ A more detailed study using D6S251 showed that 23% of sporadic BCCs exhibited LOH and MSI. All BCCs with some instability were of high histological risk (46%). However, no alterations were found in microsatellite D6S252, located at 6q16⁷⁰ (unpublished data).

Karyotype analyses of tumors demonstrate major alterations, whether in chromosome number (gain or loss) or in rearrangements between chromosomes. The analysis of certain microsatellites is also valuable in this kind of information and has been used to map chromosome regions containing genes involved in the development of BCC, where losses of regions near to the PTCH gene are particularly high.⁴²

Role of UV in the development of NMSC

Even though UVA is the most abundant (90%), UVB is about a thousand times more efficient in causing skin burns. Exposure of the skin to UV affects the survival and proliferation of epidermal and dermal

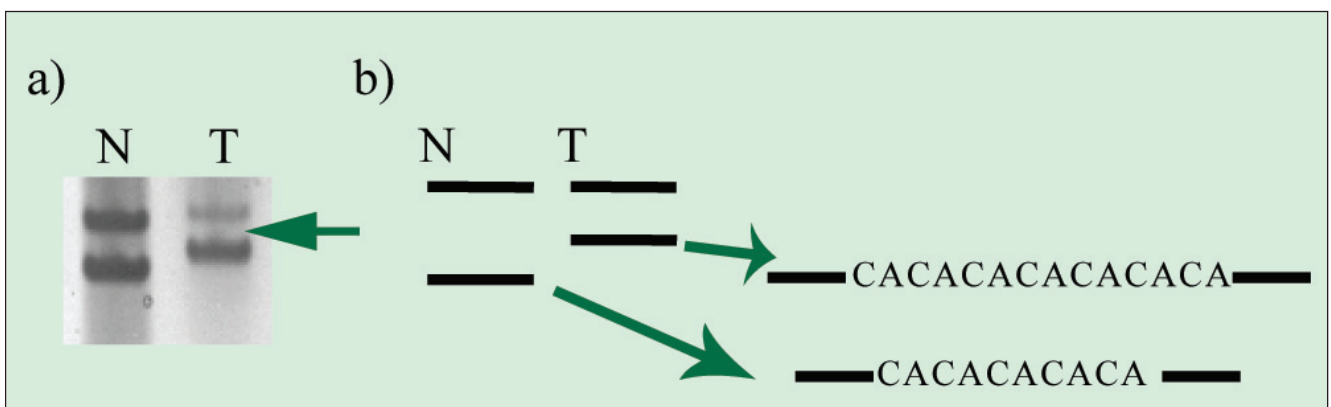


FIGURE 3: Microsatellite instability in BCC: (A) Experimental visualization of the alteration in one of the alleles of microsatellite D6S251 in BCC (modified from Francisco et al.⁶⁹). The arrow indicates the allele that suffered the alteration, showing a pattern that is different from the respective allele in the normal tissue; (B) Schematic representation of the change described. The altered pattern observed was caused by an increase in the repeats existing in the microsatellites, here in AK cases. The occurrence of such instabilities is indirect evidence, that is, a marker of other possible alterations in regions next to these microsatellites

cells and alters several skin functions.⁷¹ The acute effects of exposure to UV are the most damaging and include DNA lesions, apoptosis, erythema, immunosuppression, aging and cancer.⁷²

One of the main effects of UV in the development of cancer is the direct damage to the DNA. The absorption of UVB by the DNA can cause two types of lesions - the photoproducts 6-4 and the pyrimidine dimers or cyclobutanes. The damages are caused by a misbonding of two pyrimidines in the same DNA strand. Instead of the classical AT or GC pairing, the bases can bond through CC, TT or CT. Pyrimidine dimers are considered more carcinogenic than 6-4 photoproducts, since they form about three times more and are less efficiently repaired.⁷³ Both types of lesion can lead to genetic mutations such as C♦T and CC♦TT transitions. UVB can also cause C♦A and G♦T transversions, besides single- and double-strand DNA breaks.⁷⁴

Other kinds of lesions that can affect the DNA are those produced by reactive oxygen species (ROS) generated by excessive exposure to UV,⁷⁵ characterizing an indirect effect of radiation. These molecules can lead to formation of adducts in the bases which compose the DNA molecule, causing mismatch and thus being able to lead to chromosome mutations and rearrangements that could result in cancer.

Importance of the DNA damage repair system in NMSC

Development of skin cancers has been frequently observed in patients with XP. This disease is characterized by deleterious mutations in the genes involved in the DNA damage repair system, with a marked reduction in the ability of its cells to repair potentially carcinogenic mutations, mainly those caused by UV, thus making these patients highly photosensitive and predisposed to skin cancer. Could polymorphisms, that is, genetic variations of these genes in the population, contribute to higher susceptibility to these neoplasms?

Based on this question, many researchers have conducted epidemiologic studies to evaluate whether there is a larger occurrence of certain polymorphisms in cancer cases as compared to controls.⁷⁶ Differently from mutations, which are rare and show high penetrance and little influence of environmental factors, polymorphisms are more frequent in the population. Moreover, they function as low-penetrance factors and can present variable susceptibility to environmental risk factors, such as exposure to UV.⁷⁷

Some studies demonstrated that, while patients of Scandinavian origin with a certain genoty-

pe for the repair gene XPD (in this case, dominant homozygotes and heterozygotes)⁷⁸ were at higher risk of developing BCCs, in a US population, individuals with a homozygous recessive genotype showed higher susceptibility to this kind of skin cancer.⁷⁹ This difference demonstrates that environmental and genetic factors of a certain population have an influence in the contribution of a given polymorphism to the development of tumors.

Breaks in the structure of the DNA molecule are also a result of UV action, and such phenomena are responsible for characteristic chromosomal aberrations in skin tumors, such as BCC. Repair of these lesions is performed by another repair pathway, also formed by several genes, which also present the already described polymorphisms. Recently, it was demonstrated that the combination of certain polymorphisms increases the risk of developing SCC ($p < 0.05$) and mainly BCC ($p < 0.0001$).⁸⁰ Reinforcing this hypothesis, an in vitro study showed a significant number of chromosome breaks in lymphocytes from patients diagnosed with SCC and BCC, when exposed to UV.⁸¹

As opposed to other types of neoplasms, in which polymorphisms of repair genes demonstrated a strong associated relative risk, such studies on NMSC are scarce. Considering the variety of existing polymorphisms, well-conducted epidemiologic case-control studies, evaluating both the environmental factors involved - such as the sun exposure pattern and other risk factors - and the polymorphic variants of repair genes, could be very useful to identify possible risk groups for the development of these neoplasms.

The role of the human papillomavirus (HPV) in NMSC

Like the mutations, the action of HPV annuls the function of protein p53 in human skin cancers, a fact that has recently been the object of an extensive review.⁸² Out of more than 100 HPV subtypes identified, only a small subgroup named high-risk mucotropic HPV (HPV types 16, 18, 31, 33, 35, and 58), has been held responsible for the development of cervical cancer. The gene E6 of this high-risk HPV can induce rapid proteosomal degradation of p53, abolishing cell cycle arrest or apoptosis. As in cervix carcinomas, HPV DNA is often detected in skin carcinomas, where over 40 subtypes were identified,⁸³ but they are not high-risk.⁸⁴ It was suggested that mechanisms different from those occurring in genital cancer might be involved in the malignant neoplastic transformation of the skin.⁸⁵

Recently, HPV-38 was detected in 50% of skin carcinomas and in 10% of healthy skin samples, and

it may be responsible for the longevity/immortalization of human keratinocytes in culture.⁸⁶ Another study demonstrated that HPV-38 DNA was present in 43% of AK, as well as in 13% and 16% of SCC and BCC, respectively.⁸⁷ It is furthermore believed that several HPV types are involved in verruciform epidermodysplasia.⁸⁸

Molecular and cytogenetic alterations in NMSC

Using cytogenetic analysis and comparative genomic hybridization that allow establishing a vast panel of gains and losses,⁸⁹ SCC presented a large cytogenetic heterogeneity, with an aberration profile that is more complex than that of AK and keratoacanthomas.^{47,51,90,91} Many structural aberrations affecting centromeric regions, especially of chromosomes 3, 5, 8, and 9, were found in SCC.⁴⁷ Genetically unrelated cell clones were also observed within the same tumor, suggesting multifocal development in skin cancers. The detection of LOH in markers located on 9p is frequent, whereas on 9q it occurs in only 12% of SCCs.⁵² LOH can also occur in other regions of the genome, such as 3p, 13p, 17p, and 17q.⁴⁵

CONCLUSIONS

Despite current broaden knowledge regarding the molecular genetics of the two main types of NMSC, little is still known about the role of the countless oncogenes, suppressor genes and signal transduction pathways in the genesis and development of these neoplasms.

NMSCs present many alterations, at both the gene and the chromosome levels. The main cause of BCC development has been associated to the patched/sonic-hedgehog pathway. The use of drugs which reverse the effects of oncogenic mutations of SMO and PTCH, such as the substance cyclopamine, may help treating the several BCC phenotypes in a near future.

Understanding the mechanisms which promote genomic instability, such as gains or losses of sequences and chromosome translocations, may soon not only be of help in tumor staging, but also in the establishment of new therapies which will benefit millions of patients which are yearly diagnosed all over the world. □

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Questions and answers to questions

1. The correct concept of a gene is:
 - a) a DNA segment that obligatorily carries information for protein synthesis
 - b) a DNA segment whose primary transcription is an RNA
 - c) any DNA segment, regardless of its containing information for RNA or protein
 - d) the whole DNA base sequence

2. According to the “central dogma of molecular biology”, what can NOT happen?
 - a) RNA synthesis from DNA
 - b) DNA synthesis from RNA
 - c) Protein synthesis from RNA
 - d) Protein synthesis directly from DNA

3. The action of oncogenes in the development of tumors occurs by:
 - a) DNA damage repair;
 - b) Cell growth promotion;
 - c) Blocking of cell cycle progression;
 - d) Leading cells to death (apoptosis).

4. The table below shows four DNA sequences:

1) ATGCAGTACGAAGCAT	3) AAGCCTGCCAATTCTCCCC
2) GTAATGCTACGATAGG	4) AAGGAAACGTAGTACACGG

Based on the knowledge of the mutagenic effects of ultraviolet (UV) radiation, which sequence would have the greatest probability of presenting lesions of the pyrimidine dimer type?

 - a) 1
 - b) 2
 - c) 3
 - d) 4

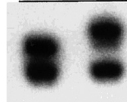
5. A young patient comes to the doctor’s office presenting several skin tumors, including BCCs, SCCs and melanoma. The patient is diagnosed as having xeroderma pigmentosum. Knowing that this is a recessive genetic disease, what genes must be mutated?
 - a) Repair genes
 - b) Oncogenes
 - c) Genes involved in cell death
 - d) Cell differentiation genes

6. During intense sun exposure, countless cells may have their DNA damaged. Which protein can contribute to the repair of these lesions or can trigger the cell death process if the repair is not efficient?
 - a) p53
 - b) Patched
 - c) BRAF
 - d) K-RAS

7. Keeping in mind the patched signaling pathway and its contribution to the carcinogenesis of BCC, what could be expected from mutations that ACTIVATE smoothened?
 - a) There would be a phenotype resembling xeroderma pigmentosum
 - b) The probability of developing BCC would decrease
 - c) The probability of developing BCC would increase
 - d) There would be no implication in the development of BCC

8. A researcher made an analysis of microsatellites from a certain chromosome region of several cases of a certain type of tumor and found a high percentage of loss of heterozygosity (LOH) in this region. This probably indicates that:
 - a) This region is under the influence of p53
 - b) This region is the site of a proto-oncogene involved in this neoplasm
 - c) This region is the site of a tumor suppressor gene involved in this neoplasm
 - d) This region does not possess genes of relevance to the progression of the tumor

9. The figure below represents a type of alteration in microsatellites.

N	T
905	
	

Knowing that normal (N) and tumor (T) tissue are represented above, we can appoint that alteration as being:

 - a) microsatellite instability (MSI), with loss of repeats by the tumor
 - b) microsatellite instability (MSI), with loss of repeats by the normal tissue
 - c) microsatellite instability (MSI), with gain of repeats by the tumor
 - d) microsatellite instability (MSI), with loss of repeats by the normal tissue

10. Recent studies have attempted to correlate the presence of certain polymorphisms in important genes to the development of some tumors. These studies have as their theoretical basis:

- a) The idea that those polymorphisms inactivate completely the activity of these genes
- b) The assumption that those polymorphisms disorganize the chromosome structure
- c) The idea that those polymorphisms are correlated with the activation of proto-oncogenes
- d) The idea that those polymorphisms contribute to a reduced activity of the gene, that normally does not cause any problem, but can – in certain situations - lead to processes such as the development of cancer

11. Genetic mutations can confer to cells certain characteristics which are important in the development of cancer. These characteristics can be represented by:

- a) Reduction in proliferation capacity, evasion of apoptosis, ability to generate metastases, and loss of response to growth inhibition factors
- b) Loss of response to growth inhibition factors, reduction of angiogenesis, evasion of apoptosis, reduction in proliferation capacity
- c) Increase of apoptosis, greater response to growth inhibition factors, ability to generate metastases
- d) Increase in proliferation capacity, evasion of apoptosis, ability to generate metastases, and loss of response to growth inhibition factors

12. The genes considered as tumor suppressor genes are:

- a) RB1, MYC, CDKN2A and TP53
- b) PTCH, BCRA1, RB1 and CDKN2A
- c) BRAF, PTCH, CDKN1A and ABL
- d) TP53, RB1, K-RAS and ABL

13. Which is the main function of protein p16?

- a) To inhibit p53 in phase G2 of the cell cycle
- b) To impair progression toward phase G1, blocking CDK-4
- c) To activate p53 by bonding to mdm2
- d) To activate CDK-4 in rb phosphorylation

14. The action of proto-oncogenes in tumor growth is due to:

- a) Activation during the embryonic phase taking advantage of the cellular vigor of young organisms
- b) Direct action on the activation of apoptosis, besides keeping protein RAS activated
- c) As they are transformed into oncogenes, they express themselves by signaling uncontrolled cell multiplication
- d) They are genetically dominant over the tumor suppressor genes

15. Codon 278 of gene TP53 is considered a hotspot because that is where:

- a) more specific BCC mutations occur
- b) more specific SCC mutations occur
- c) less specific BCC mutations occur
- d) more specific melanoma mutations occur

16. What is the function of the HPV in human skin cancers?

- a) To annul the function of protein p53
- b) To activate tumor suppressor genes
- c) To encode oncogenes
- d) To activate the patched gene

17. The suggestion of multifocal development in skin cancers is due to:

- a) The presence of different genes in different cells of the same tumor
- b) The arising of genetically identical metastases
- c) The observation of genetically unrelated cell clones within the same tumor
- d) The contamination by various HPV strains

18. The pre-mRNA processing function during splicing is important to:

- a) Remove the exons and link the introns, necessary for protein synthesis
- b) Remove the amino acids which do not belong to the protein
- c) Excise amino acid encoding nucleotides
- d) Remove the introns and link the exons, necessary for protein synthesis

19. In the process named translation,
- a) mRNA is translated into proteins in the ribosome
 - b) rRNA transforms mRNA into proteins
 - c) DNA is processed into proteins
 - d) tRNA is translated into proteins in the ribosome
20. The main alterations in SCC development include:
- a) loss of 6q, mutations in TP53
 - b) LOH in D6S251 and mutations in TP53
 - c) loss of 9p and mutations in TP53 and CDKN2A
 - d) MSI in D6S251 and mutations in TP53

ANSWERS

Skin manifestations of illicit drug use. *An Bras Dermatol.* 2006;81(4):307-17.

- | | |
|-------|-------|
| 1. c | 11. a |
| 2. b | 12. a |
| 3. b | 13. b |
| 4. d | 14. b |
| 5. d | 15. d |
| 6. a | 16. c |
| 7. b | 17. a |
| 8. b | 18. b |
| 9. d | 19. b |
| 10. c | 20. b |