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Review Article

Role of *p53* and *CDKN2A* Inactivation in Human Squamous Cell Carcinomas

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p53 tumor suppressor gene is the most commonly mutated gene in human and mouse cancers. Disruption of the *p53* and *Rb* pathways is a fundamental trend of most human cancer cells. Inactivation of *CDKN2A* can lead to deregulation of these two pathways. Genetic abnormalities in *CDKN2A* gene have been well documented in human melanoma but their involvement in human nonmelanoma skin cancer (NMSC) and in particular in squamous cell carcinoma (SCC) is less clear. Several studies have shown that human SCCs harbour unique mutations in the *p53* gene as well as inactivation of the *CDKN2A* gene. While mutations in the *p53* gene are induced by UV radiation and represent tumor initiating events, the majority of alterations detected in the *CDKN2A* gene do not appear to be UV-dependent. In conclusion, in addition to *p53* mutations, silencing of the *CDKN2A* gene might play a significant role in SCC development.

1. INTRODUCTION

Cutaneous SCC is the second most common malignancy diagnosed in Caucasian population. Its incidence varies considerably and is reported to be increasing worldwide [1]. SCC arise on sun-exposed areas of the skin and may behave aggressively, resulting in recurrence or metastasis [2].

Many genetic and environmental factors are known to contribute to the development of SCCs, the most important being repeated exposures to the ultraviolet (UV) in the sunlight [3, 4]. Epidemiological studies clearly showed a correlation between repeated exposure to UV radiation in childhood and an increased incidence of skin cancer especially in Caucasians with fair skin [5].

Wavelengths in the region (290–320 nm) of the solar spectrum are absorbed into the skin producing erythema, burns, and eventually skin cancer. Laboratory studies have shown that UVB region of the solar spectrum is responsible for these effects [6]. Chronic UV exposure may cause mutations in cellular DNA unless photoproducts are repaired, and the accumulation of genetic abnormalities leads to tumor formation [7].

It is widely accepted that SCCs develop through a multistep process that involves the activation of protooncogenes and/or inactivation of tumor suppressor genes. The initial damage takes place in the DNA after which DNA repair is undertaken by a complex array of gene repair proteins [8].

Several studies have shown that SCCs harbour unique mutations in the *p53* tumor suppressor gene that are not commonly found in other human cancers. These mutations termed UV signature mutations, consist of single (C → T) and double (CC → TT) pyrimidine base substitutions and have been identified either in premalignant or in malignant cutaneous squamous lesions [9]. In fact, the finding that *p53* mutations are present in actinic keratosis (AK) and in sun-damaged skin suggests that *p53* mutations arise early during the development of SCC [10]. This hypothesis is supported by laboratory studies demonstrating that clones of keratinocytes with mutant *p53* protein and *p53* mutations can be detected in UV-irradiated mouse skin well before the appearance of skin tumors [11, 12]. The presence of UV signature mutations at dipyrimidine sites of the *p53* gene indicates strongly the role of UV radiation in skin carcinogenesis.

Disruption of the *p53* and *Rb* tumor suppressor pathways is a fundamental trend of most human cancer cells. In tumorigenesis, loss of *Rb* function can occur by direct inactivation of the *Rb* gene itself through mutation or by deregulation of the genes controlling *Rb* phosphorylation status. These last alterations include cyclin-D1 gene amplification, *CDK4* activating mutations, and also gene amplification

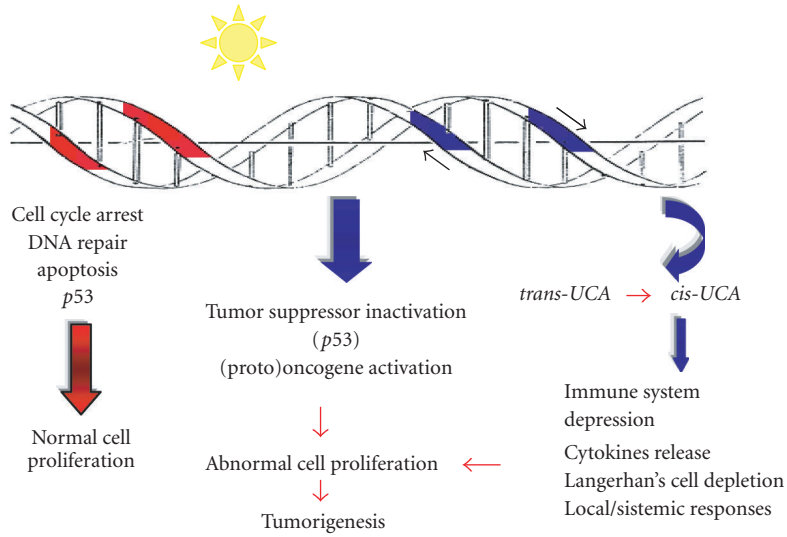

Figure 1: Molecular events following UV exposure.

and inactivation of the inhibitors of *CDK4*, the *INK4* family [13, 14].

The *CDKN2A* (INK4a/ARF) locus at *9p21* encodes two alternatively spliced proteins, $p16^{INK4a}$ and $p14^{ARF}$, functioning as cell cycle inhibitors [14]. Several studies have shown that a subset of SCCs of the skin carries mutations in the *CDKN2A* tumor suppressor gene [15]. Although frequent inactivation of *CDKN2A* has been reported in SCCs from xeroderma pigmentosum patients [16], its involvement in sporadic SCCs is not completely understood yet. The studies conducted by Soufir et al. and Brown et al. showed inactivation of *CDKN2A* in the 24% and 76% of SCCs, respectively, (see [3, 17]).

2. *p53* INACTIVATION

The *p53* tumor suppressor gene encodes for *p53*-kd nuclear phosphoprotein, which has been named "guardian of the genome." It functions as a regulator of cellular responses to genotoxic injury, including exposure to ultraviolet radiation. It arrests cell division at G1 phase to allow DNA repair. In particular, it has been demonstrated that in this pathway $p21^{WAF1/CIP1}$ acts as an inhibitor of the cyclin-dependent kinase (CDK) whose induction is associated with the expression of *p53* [18]. *p21* mediates cell cycle arrest by binding to and inactivating the cyclin D/CDK4, cyclin D/CDK6, and cyclin E/CDK2 complexes. When D-type cyclins are complexed with CDK4/6 phosphorylate serine and threonine residues on the retinoblastoma (Rb) protein, this tethers the *Rb* from E2F transcriptional factors, thereby enabling the E2F-mediated activation of a series of target genes essential for S phase entry. The overexpression of *p21*, however, causes the accumulation of hypophosphorylated Rb (pRb) and the sequestration of E2F, which causes the cell to be arrested in G21 phase [19]. If the genomic insult is extensive instead, *p53* induces apoptosis in an effort to eliminate potentially transformed cells (Figure 1). Inactivation of the *p53* gene, either by mutation or other mechanisms, results in an increased rate of accumulation of genetic damage in cells and promotes tumor formation [20].

In normal conditions, a very small amount of *p53* protein is present in cells; in response to DNA damage, protein accumulates, cell division is inhibited, and DNA repair occurs. It is thought that inhibition of cell division enables the cell to repair damaged DNA before undergoing replication [21]. The *p53* gene is a common target for genetic alteration in human and mouse cancers and often specific carcinogens induce specific mutations in this gene [22].

Following chronic UV exposure, mistakes associated with DNA repair and replication can result into mutations in the *p53* gene, especially C → T and CC → TT transitions at dipyrimidine sites, considered as UV molecular signature. The *p53* mutation in keratinocytes is probably an initiating event in UV skin carcinogenesis [23].

As UV signature mutations in the *p53* gene are already present in benign precursor lesions of squamous cell carcinomas (AK), they appear to be an early step in the UV carcinogenesis. In the experiments with hairless mice, microscopic clusters of epidermal cells overexpressing mutant *p53* occur long before skin carcinomas become visible [11]. Such clusters are also found in sun-exposed human skin [10]. Most of human non melanoma skin cancer are found to bear mutations in the *p53* gene. Brash et al. [24] showed *p53* mutations in 58% of human SCCs analyzed (3/24 showed CC → TT transition and 5/24 had C → T base change) [22]. In a recent study, Bolshakov et al. analyzed 342 human NMSC and found *p53* mutations in 28/80 aggressive SCCs and in 28/56 non aggressive SCCs. About 71% of the detected *p53* mutations were UV signature mutations [25].

Experiments to determine the timing of *p53* mutation in relation to skin cancer development have been performed in the mouse model of photocarcinogenesis because this

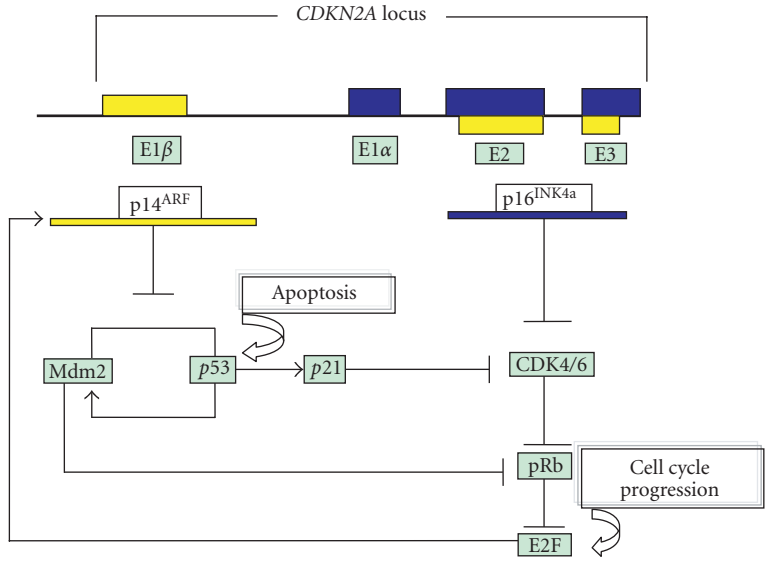

Figure 2: Genomic organization of the *CDKN2A* locus and description of involvement of its transcripts ($p16^{INK4a}$ and $p14^{ARF}$) in the *Rb* and *p53* pathways. $p16^{INK4a}$/CDK4/pRb pathway: CDK 4 negatively regulates pRb protein activity by phosphorylation. $p16^{INK4a}$ inhibits CDK4 binding to cyclin D preventing inactivation of pRb. Hypophosphorylated pRb represses E2F-dependent genes blocking G1/S cell cycle progression. $p14^{ARF}$/p53 pathway: *p53* is targeted by MDM2 for ubiquitination and degradation. In response to oncogenic signals, $p14^{ARF}$ induces MDM2 molecular relocalization, thereby preventing MDM2-p53 interactions and resulting in the stabilization of *p53*.

model is easily referable to UV irradiation and sample collection at various time points during the carcinogenesis protocol. Ananthaswamy et al. have shown that *p53* mutations in mouse skin arise very early during UV carcinogenesis by using a very sensitive mutation-specific PCR technique for the early detection of *p53* mutations in UVB-irradiated C3H mice. They were able to detect *p53* mutations at 4th week of UV irradiation and the frequency of *p53* mutations increased progressively and reached 50% at 12th week of chronic UV exposure. The real *p53* mutations frequency observed in this study is probably higher than the one described because only tandem CC → TT mutations were analyzed [12]. In addition to C3H mice, Ouhtit et al. used SKH-hr1 mice to determine the timing of *p53* mutations during UV carcinogenesis. Interestingly, *p53* mutations were detected as early as 1st week of chronic UV irradiation and the mutation frequency reached 80%–90% by 4–8 weeks [26]. Both the early appearance and the high frequency of *p53* mutations in UV-irradiated SKH-hr1 mice skin can be explained with the fact that either C → T or CC → TT mutations were analyzed in this study.

In a recent study, Melnikova et al. investigated whether discontinuation of UV exposure before the onset of skin tumors results in the disappearance of *p53* mutations in the skin of SKH-hr1 mice. Their results indicated that despite discontinuation after 8 weeks, UV irradiation results in 100% skin tumor incidence, although the kinetics of tumor occurrence is greatly delayed. In terms of human relevance these results suggest that early life exposure to UV may introduce *p53* gene mutations in epidermal keratinocytes as well as keratinocytes progenitors. While some *p53*-mutated keratinocytes may be eliminated via differentiation and epidermal desquamation, others, may still persist and eventually give rise to skin tumors even in the absence of further UV exposure. Thus, cancer development can be delayed but not definitively abrogated upon further avoidance of exposure to UV radiation [27].

3. *CDKN2A* INACTIVATION

The presence of *p53* mutations in sun-exposed skin and premalignant lesions suggest that *p53* mutations arise early and may be required but not sufficient for tumor development. Therefore, it is expected that additional genetic alterations in oncogenes and tumor suppressor genes are essential for the development of SCCs.

The *CDKN2A* locus at *9p21* (Figure 2) is frequently inactivated in human cancers and it consists of two overlapping genes that encode two unrelated proteins, $p16^{INK4a}$ and $p14^{ARF}$, functioning as cell cycle inhibitors.

$p16^{INK4a}$ and $p14^{ARF}$ share the same exon 2 but having a distinct exon 1, exon 1α and exon 1β, respectively. Because exon 1β splices into common exons 2 and 3 in a different reading frame, the resulting $p14^{ARF}$ bears no similarity to $p16^{INK4a}$. It is well established that $p14^{ARF}$ plays a role in cell cycle control linking the $p16^{INK4a}$/Rb pathway and the *p53*/*Rb* pathway. Upon phosphorylation of *Rb*, E2F is activated and promotes induction of $p14^{ARF}$, which in turn sequesters MDM2 and thereby prevents degradation and nuclear export of *p53*. Both $p16^{INK4a}$ and $p14^{ARF}$ transcripts by their interactions with pRb and *p53* are important in regulating the proliferation of normal and tumorigenic squamous epithelial cells [28, 29].

Inactivation of the tumor suppressor gene *CDKN2A* can occur in a variety of genetic mechanisms including mutations and deletions. In addition, hypermethylation of the CpG islands of gene promoter is an effective means of gene silencing in a variety of tumors. Inactivation of *CDKN2A* by deletion, mutation, or promoter hypermethylation in a wide range of malignancies has been documented [30].

It has been shown by Soufir et al. that SCCs from xeroderma pigmentosum patients contain mutations in *CDKN2A* gene in 13/28 SCCs and 54% of mutations detected at *CDKN2A* locus were UV signature mutations [4].

In order to determine the involvement of *CDKN2A* gene in sporadic SCCs, Saridaki et al. performed the allelic imbalance analysis and the mutational analysis on 22 SCCs and on 5 Bowen's disease specimens. Their results indicated that 52% of specimens exhibited loss of etherozygosity (LOH) in at least one microsatellite marker whereas only 2/27 samples exhibited microsatellite instability. Mutational analysis revealed the presence of a base substitution in exon 1α of 1 tumor and the presence of a C → T transition in exon 2 in a second tumor [31].

Brown et al. analyzed 30 cutaneous SCCs from 29 patients immunosuppressed and 10 tumors from immunocompetent patients and have shown that the total frequency of *9p21* alterations was 76%, with abnormalities of $p16^{INK4a}$ detected in 53% of tumor analyzed and of $p14^{ARF}$ in 64% of the tumors. Promoter methylation was the predominant mechanism of inactivation for both genes. Biallelic events were common [17].

Murao et al. examined the epigenetic abnormalities of a wide range of cancer-related genes (*CDH1*, *p16*, *p15*, *RB1*, *p*14, *DAPK1*, *MGMT*, *RASSF1*, *PTEN*, *PRDM2*, and *p53*) in 20 sporadic SCCs from 20 immunocompetent patients. Their results showed that although the frequency of methylation of $p16^{INK4a}$, Rb1, and $p14^{ARF}$ was not high, methylation of these genes in combination with mutation analysis of *CDKN2A* and *p53* revealed that 70% of cases had abnormalities of the RB1/p16 and/or *p53* pathway through either genetic or epigenetic mechanisms, except for epigenetic abnormalities of *p53* itself [32].

All these findings emphasize the importance of *CDKN2A* tumor suppressor gene in the pathogenesis of SCC.

4. CONCLUSIONS

UV radiation present in the sunlight is a potent carcinogen. Recent advances in cellular and molecular biology have clarified some of the mechanisms of photocarcinogenesis including the formation of DNA photoproducts, DNA repair, mutation of protooncogenes, and tumor suppressor genes. It is well established that UV radiation induces mutations in the *p53* gene and that these mutations arise very early during photocarcinogenesis.

In addition to mutations in the *p53* tumor suppressor gene, genetic alterations in *CDKN2A* gene leading to loss of expression of $p16^{Ink4a}$ and $p14^{ARF}$ proteins may also play an important role in the development of human NMSC.

Several studies have shown that human SCC harbor unique mutations in the *p53* gene as well as inactivation of the *CDKN2A* gene. While mutations in the *p53* gene are induced by UV radiation and represent tumor initiating events, the majority of the alterations detected in the *CDKN2A* gene do not appear to be UV dependent. Probably these genetic alterations arise spontaneously, probably during tumor progression.

In conclusion, from results of several recent studies we can assume that mutations in *p53* and *CDKN2A* genes may contribute to the initiation and progression of UV-induced skin tumors.

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