Role of \( p53 \) and \( CDKN2A \) Inactivation in Human Squamous Cell Carcinomas

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Received 15 December 2006; Accepted 26 February 2007

Recommended by Honnavara N. Ananthaswamy

\( p53 \) 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
and inactivation of the inhibitors of CDK4, the INK4 family [13, 14].

The CDKN2A (INK4a/ARF) locus at 9p21 encodes two alternatively spliced proteins, p16INK4a and p14ARF, 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 p21WAF1/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 G2 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
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 1a 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 p16INK4a detected in 53% of tumor analyzed and of p14ARF 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, p14, 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 p16INK4a, Rb1, and p14ARF 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 p16INK4a and p14ARF 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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