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REVIEW

The role of p53 in the immunobiology of cutaneous squamous cell carcinoma

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SUMMARY

Cutaneous squamous cell carcinoma is typically characterized by the over-expression of the tumour suppressor protein p53. Considerable evidence suggests that immune competence is important in the control of cutaneous SCC. We discuss the immunobiology of p53 and its relevance to cutaneous SCC, including the potential interaction with human papillomavirus.

Keywords squamous cell carcinoma p53 cytotoxic T lymphocytes human papillomavirus

INTRODUCTION

Non-melanoma skin cancer is the most common cancer amongst Caucasians with cutaneous squamous cell carcinoma (SCC) comprising approximately 20% and carrying a significant risk of metastasis [1]. Exposure to ultraviolet (UV) radiation is believed to be a major aetiological cofactor in the development of cutaneous SCC with other risks including ionizing radiation, chemical carcinogens, and viral infection (see below [1]). Immunosuppression also significantly increases the risk of cutaneous SCC development, for example 20 years after renal transplantation the risk of skin cancer development approached 40% [2].

THE IMMUNE RESPONSE TO p53

Up to 90% of cutaneous SCC lesions have UV-induced signature mutations, such as the formation of thymidine dimers, in the p53 gene [3,4]. p53 normally functions in cell cycle arrest, DNA repair and apoptosis, thus mutations in p53 may result in the unrestrained proliferation of keratinocytes (Fig. 1). During the early stages this may manifest as actinic keratoses which can then possibly progress to cutaneous SCC. Indeed some authors suggest that actinic keratoses represent *in situ* cutaneous SCC [5,6]. It has been observed that mutant p53 accumulates in the cell cytoplasm, probably due to increased half-life of the protein [7,8] and over-expression of p53 in squamous epithelium correlates with sun exposure [9,10]. In nonmalignant tissue, the ubiquitin-proteasome pathway rapidly degrades wild-type p53 [11] and the high levels of p53 expression in cutaneous SCC and other tumours contrasts to the low levels of p53 found in normal tissues.

Identification of tumour-associated antigens (TAA) remains a major goal of tumour immunology. Once identified, vaccines may be designed to induce immune response against the appropriate TAA. Interest in p53 as a TAA stems from the observation that it is over-expressed in a wide variety of tumour types and therefore a vaccine against p53 could potentially be used in a broad range of malignancies. In cutaneous SCC, as the majority of the mutations are missense point mutations, over-expression of the mutant protein may alter MHC presentation of wild-type epitopes. Induction of an immune response directed against p53 may therefore enhance tumour rejection in cutaneous SCC. However, in order for p53 to be used for therapeutic intervention, a number of issues must be addressed.

Down-regulation and mutation of TAA is one mechanism whereby tumours may be able to evade the immune system. Tumours from patients with head and neck SCC in whom p53-specific cytotoxic T lymphocytes (CTL) were present appeared to have lower expression of p53 compared to those patients in whom no p53-specific CTL were detected. In addition, some tumours were found to have mutations within or adjacent to an immunodominant epitope of p53 [12,13]. Processing and presentation of this immuno-dominant epitope can be blocked by mutation of a flanking amino acid situated on a mutation hotspot of p53 [14]. These observations suggest immune evasion by tumours can take place, possibly by outgrowth of tumours expressing low levels of p53 or p53 mutated in or around immunodominant epitopes. An immune response to p53 may enhance this kind of immune evasion.

A further difficulty in the attempt to induce immune responses against p53 arises from the fact that, as a self-antigen, p53 specific CD4 and CD8 T cells may be tolerant, or of low

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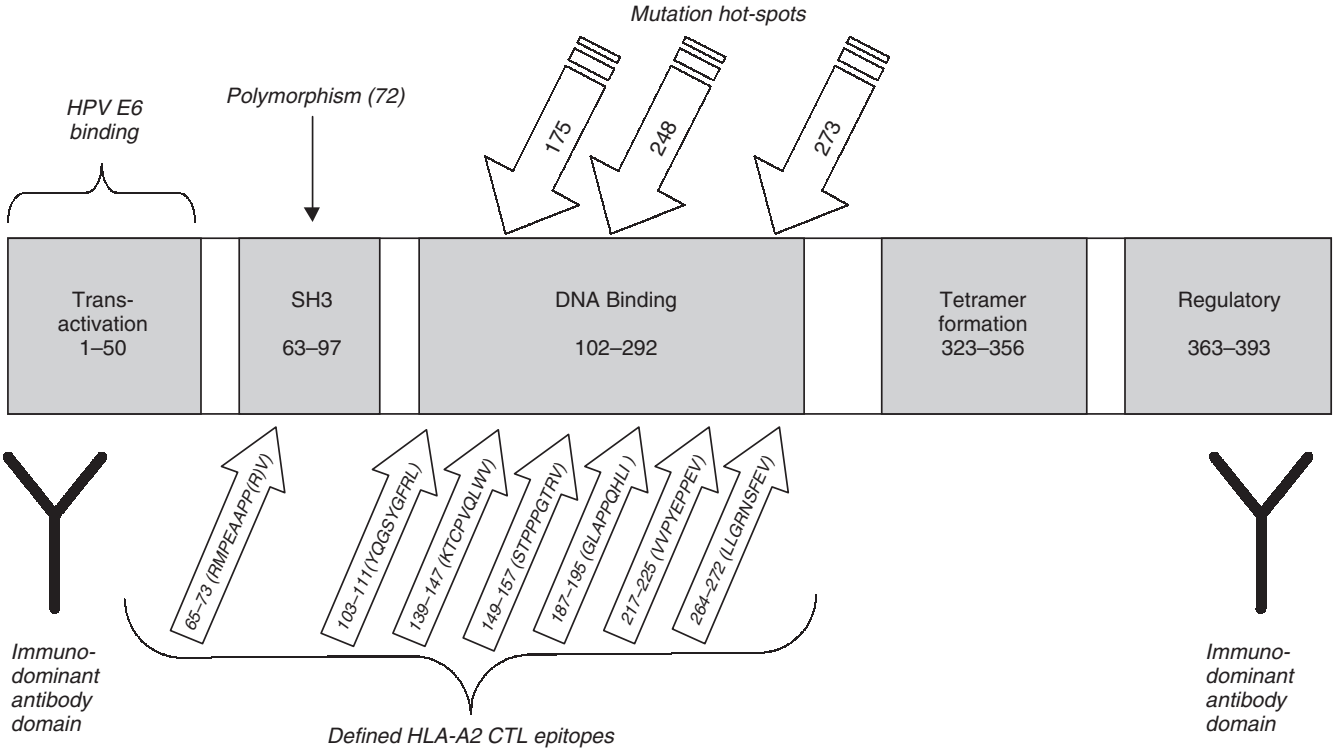

Fig. 1. p53 CTL epitopes and functional domains. Mutational hotspots are those most commonly found in skin cancer (p53 mutation database; *http://perso.curie.fr/tsoussi*). Epitopes shown are those defined for CTL recognizing peptides presented on HLA-A2 MHC class I with numbers representing amino acids [18,20,25–31].

affinity. p53-specific CTL generated from normal (p53+/+) mice have an avidity 10-fold lower than those generated from p53-/– mice probably due to deletion of high avidity p53-specific CTL in the normal mice [15,16]. Significantly, it is possible to induce increased number (but not avidity) of p53-specific CTL by eliciting CD4 T cell help and blocking CTLA-4 whilst immunizing mice with p53 peptide [17]. Immune nonresponsiveness to p53 may also be overcome by use of modified p53 epitopes that are more efficient than wild-type epitopes for inducing CTL responses to p53 [18–21].

It remains unclear whether there is a protective antip53 response in cancer patients. Antibodies against p53 are rare in normals but may occur in up to 30% of patients with cancer [8]. Mice injected with p53 expressing tumours also develop antibodies to p53, possibly due to necrosis of tumour and release of p53 into the serum [22]. The protective value of these antibodies is not known. Also, the relevance of antip53 antibodies to cutaneous SCC is unclear as one recent study found that, despite the high expression of p53 in tumours, patients with cutaneous SCC had low prevalence of antip53 antibodies [23]. The authors suggested that the antip53 response might be inhibited by UV-induced immuno-suppression.

Finally, it has been shown that tumour recognition and lysis may not be dependent on the level of p53 expression but rather on its rate of turnover. Those tumour cells with a high rate of p53 degradation may generate a high number of epitopes for recognition by T cells [24].

Despite these caveats, research into the immune response to p53 and its potential for therapeutic application has yielded promising results. Many studies have focused on the CD8 cytotoxic T cell (CTL) response to p53 and a number of human CTL epitopes have been described (Fig. 1.) [18,20,25–31]. Human CTL lines and clones specific for epitopes within p53 have been derived from the peripheral blood of normal individuals and cancer patients. Importantly, p53 specific CTL have been shown to recognize and kill a variety of tumour cell lines including SCC lines [32–38].

Little is known about the CD4 response to p53 in cutaneous SCC or other cancers. CD4 T cells from normal individuals have been shown to recognize peptides derived from the core domain of p53 [39] and peptides generated from mutant and cryptic p53 sequences also induce CD4 T cell response [40]. Interestingly, there is a proliferative response to p53 protein in patients with breast cancer who have p53 antibodies but not normals or those without antibodies [41]. In contrast, peripheral blood mononuclear cells from a cohort of colorectal cancer patients without p53 antibodies also displayed proliferative responses *in vitro* to recombinant p53 and peptides [42]. These observations have implications for cutaneous SCC given that CD4 T cells infiltrate the tumours and have been suggested to play a role in tumour regression [43,44].

p53-specific CTL capable of mediating protective immunity to tumours have been generated in a number of murine models. Adoptive transfer of p53-specific CTL generated in p53-/– mice confers immunity on the recipient to p53 overexpressing murine tumour [45]. p53-specific CTL generated in normal mice by vaccination with peptide also display antitumour immunity when adoptively transferred into recipients with established pulmonary

metastasis [46]. The use of HLA-A2 transgenic mice has demonstrated the feasibility of generating protective CTL responses to epitopes within human p53. CTL generated in these mice following peptide vaccination were able to lyse a number of human tumour cell lines [47] and also confer immunity to pancreatic tumour following adoptive transfer to nude mice *in vivo* [48].

Canarypox virus vectors expressing mutant or wild-type p53 conferred immunity on BALB/c mice challenged with a mouse fibroblast tumour cell line that expressed high levels of p53 [22]. Other vaccines used to induce p53-specific immune responses in mice include peptide pulsed dendritic cells [49], recombinant adenovirus transduced dendritic cells [50,51], recombinant DNA [52] and recombinant vaccinia virus [53].

Concerns about the safety of inducing immune responses to self-antigen have been addressed by immunization of macaques with recombinant canary pox (ALVAC) expressing p53 protein. This regimen does not induce adverse autoimmunity despite the appearance p53 antibodies [54]. More recently, a recombinant ALVAC-p53 vaccine has been used to successfully induce antip53 T cell responses, without obvious autoimmune symptoms, in end-stage colorectal cancer patients [55]. These data contrast to another recent study in which p53-recombinant adenoviral vaccination did not generate p53-specific CTL or antibodies in six cancer patients [56]. These studies may be relevant to cutaneous SCC given the high levels of expression of p53 in the SCC lesions.

INTERACTIONS OF p53 WITH HUMAN PAPILLOMAVIRUSES

Human papillomaviruses (HPV) are believed to be additional cofactors in the development of cutaneous SCC. Interaction with p53 may prove to be a mechanism by which HPV may alter the immunobiology of cutaneous SCC.

HPV are nonenveloped viruses with double stranded DNA approximately 5kb in size. They infect epithelial cells of the skin and mucosa and replicate in differentiating keratinocytes. The viruses consist of seven early proteins (E1-E7) and 2 late structural proteins (L1 and L2). HPV types are defined according to DNA homology and over 80 types have been described but are difficult to culture *in vitro* – possibly due to their inability to grow differentiated keratinocytes (reviewed in [57]).

A link between HPV infection and cervical cancer has been established for a long time. HPV16 and HPV18 – the 'high risk' types – are commonly found in cervical cancer lesions. The transforming properties of HPV 16 are mediated by the E6 protein that disrupts p53 expression [58] and the E7 protein that interferes with the retinoblastoma tumour suppressor protein [59,60]. Human vaccine trials with cervical cancer patients are currently underway using vaccines designed to induce immune response to high risk HPV (reviewed in [61]).

The role of HPV infection in the development of cutaneous SCC is less well established. Evidence comes from the link between HPV infection and cutaneous SCC development in epidermodysplasia verruciformis (EV) patients and immunosuppressed patients. EV is an autosomal recessive disease in which warts and macular lesions are widespread on the body, particularly on sun-exposed skin. The disease is associated with a high risk of nonmelanoma skin cancer – approximately one third of EV patients develop cutaneous SCC. A susceptibility to infection with specific HPV types (such as HPV 5, 8, 20, 21, 23, 24, 38) is thought to be responsible for the high occurrence of warts and malignancies in EV [62,63].

Immunosuppressed patients, such as transplant recipients, show up to 100 fold increased susceptibility to development of cutaneous SCC on sun-exposed skin [64]. Although the HPV types vary according to the detection method used, it is believed that there is a high prevalence of EV type HPV in the lesions of these patients [65–68].

Some of the inconsistencies associated with detection of HPV DNA are due to use of different primers by different investigators [69]. Such problems have been overcome by the employment of a degenerate PCR technique, making use of nested primers that are specific for a broad range of HPV types [70,71] (reviewed in [72]). These studies have confirmed the presence of multiple HPV types, predominantly EV-types, in lesions from EV and immunosuppressed patients as well as immunocompetent patients with cutaneous SCC. However, no particular HPV type appears to be strongly associated with cutaneous SCC. Detection of HPV DNA in the skin of normal individuals also questions the significance of the presence of HPV in cutaneous SCC [73]. There have been few serological investigations into the role of HPV infection in cutaneous SCC. One study found an increased prevalence of antibodies against the EV-associated HPV 8 in individuals with actinic keratoses [74].

As mentioned above, E6 protein from the cervical associated HPV-16 mediates degradation of p53. A common p53 polymorphism at position 72 replacing proline with arginine renders p53 more susceptible to E6 mediated degradation. The arginine allele was found to be a risk factor in the development of cervical cancers and there was also a significant association with cutaneous SCC development in renal transplant patients [75]. The two allelic variants of p53 also differ in their ability to induce apoptosis following DNA damage [76]. These findings stimulated investigations into the association of the p53 polymorphism and cutaneous SCC. Unfortunately, conflicting data has clouded the results of these studies, possibly due to interlaboratory variation in protocols [77]. Some groups confirmed an association of the polymorphism with development of cutaneous SCC in immunosuppressed patients [78] whilst others have found no association with the development of cutaneous SCC [79–82].

The presence of UV-induced p53 mutations in cutaneous SCC tumours contrasts with tumours induced by 'high risk' HPV types, which contain wild-type p53. It remains a tantalizing possibility that the arginine allele of p53, perhaps in combination with UV induced mutation, is more susceptible to interference from particular HPV types and subsequent malignant transformation.

Lastly, sunlight may play a role in the pathogenesis of cutaneous SCC other than the induction of p53 mutation. HPV 77, a type only so far detected in cutaneous lesions of renal transplant patients, contains a p53-DNA binding site. Once activated by UV, p53 may stimulate HPV 77 promoter activity [83]. Also, UV induced pro-inflammatory cytokines released by keratinocytes can regulate promoter activity of HPV 20 and HPV27 [84].

CONCLUSIONS

Overall there is significant evidence supporting a role for CD8+ T cells in the control of cutaneous SCC and induction of a specific CTL response is an important aim of tumour vaccination (reviewed in [85]). As p53 is over-expressed in the majority of

cutaneous SCC, vaccination against p53 is an appealing strategy to induce tumour-reactive immunity. This may be particularly relevant for individuals with recurrent or metastatic cutaneous SCC but may also be considered in individuals thought to have a high risk of primary SCCs. Several problems would need to be overcome in order to generate protective immunity using a p53 vaccine. Whilst studies showing loss of p53 CTL epitopes in human cancer patients [12,13] and down-regulation of p53 in ALVAC-p53 immunized mice [22] argue for the significance of p53 specific CTL, they also underline the problem of escape mutations and tumour evasion. However, down-regulation of p53 by a tumour may diminish a dominant negative effect of a p53 missense mutation and thus represent a selective disadvantage for the tumour. Furthermore, vaccination with multiple epitopes or whole protein, concurrent cytokine vaccination, use of p53 variant peptides and/or dendritic cell therapies may overcome these difficulties. The roles of HPV in the aetiology of cutaneous SCC and their interactions with p53 have yet to be fully characterized. The development of techniques to detect HPV-specific T cell responses in an HPV-type independent manner will contribute to our understanding of their role in cutaneous SCC pathogenesis and therapeutics.

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