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The multi-step process of human skin carcinogenesis: A role for p53, cyclin D1, hTERT, p16, and TSP-1

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Abstract

As proposed by Hanahan and Weinberg (2000. Cell 100, 57–70) carcinogenesis requires crucial events such as (i) genomic instability, (ii) cell cycle deregulation, (iii) induction of a telomere length maintenance mechanism, and (iv) an angiogenic switch. By comparing the expression of p53, cyclin D1, p16, hTERT, and TSP-1 in spontaneously regressing keratoacanthoma (KA) as a paradigm of early neoplasia, with malignant invasive cutaneous squamous cell carcinoma (SCC) as a paradigm of advanced tumour development, we are now able to assign the changes in the expression of these proteins to specific stages and allocate them to defined roles in the multi-step process of skin carcinogenesis. We show that mutational inactivation of the p53 gene, and with that the onset of genomic instability is the earliest event. Individual p53-positive cells are already seen in "normal" skin, and 3/5 actinic keratoses (AKs), 5/22 KAs, and 13/23 SCCs contain p53-positive patches. Cell cycle deregulation was indicated by the overexpression of the cell cycle regulator cyclin D1, as well as by the loss of the cell cycle inhibitor p16. Interestingly, overexpression of cyclin D1 – observed in 80% of KAs and SCCs, respectively – showed a cell cycle-independent function in HaCaT cell transplants on nude mice. Cyclin D1 overexpression was associated with a massive inflammatory response, finally leading to tissue destruction. Loss of the cell cycle inhibitor p16, on the other hand, correlated with SCCs. Thus, it is tempting to suggest that overexpression of cyclin D1 is an early change that in addition to growth stimulation leads to an altered epithelial–mesenchymal interaction, while functional p16 is able to control this deregulated growth and needs to be eliminated for malignant progression. Another requirement for uncontrolled growth is the inhibition of telomere erosion by up-regulating telomerase activity. As measured by hTERT protein expression, all of the KAs and SCCs studied were positive, with a similar distribution of the protein in both groups and an expression pattern resembling that of normal epidermis. Thus, telomerase may not need to be increased significantly in skin carcinomas. Finally, we show that the angiogenesis inhibitor TSP-1 is strongly expressed in most KAs, and mainly by the tumour cells, while in SCCs the generally weak expression is restricted to the tumour–stroma. Furthermore, we provide

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evidence that the loss of a copy of chromosome 15 is responsible for reduced TSP-1 expression and thereby this aberration contributes to tumour vascularisation (i.e. the angiogenic switch) required for malignant growth.

Keywords: Keratoacanthoma; Squamous cell carcinoma; P16; P53; Thrombospondin; Cyclin D1

Introduction

Skin cancer, comprising basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and malignant melanoma (MM), is the most frequent cancer worldwide. While BCC and MM are extensively studied, knowledge about the development of SCC is still fragmentary (for review see Boukamp, 2005a). This is unfortunate considering that SCC has become an increasing burden for the general population, and that it is particularly life threatening in immunosuppressed patients. In these individuals the incidence of SCCs is significantly (250-fold) increased, but most importantly, the overall metastasis rate is approaching 10%, (10-fold higher than that seen in the immunocompetent population). In view of such alarming rates and disease outcomes, it is more important than ever to better understand the stage-specific, regulatory molecular–genetic changes involved in skin cancer progression, and to better correlate these changes with altered cellular functions.

SCCs develop at sites of chronic UV damage (preferentially located on the face, head, neck, back, hand, and forearm). Since the prevalence also correlates with age, chronic sun exposure is believed to be the major carcinogenic risk factor. As the metastatic rate is the highest (up to 50%) in SCC of the lip – one of the most richly vascularised anatomical sites – it is tempting to hypothesise that, besides control by the immune system, an increased blood vessel supply is a determining risk factor in malignant progression.

Skin carcinogenesis, as in most other cancer types, is believed to involve a multi-step process with early actinic keratosis (AK) and full thickness carcinoma in situ representing about 10% of the precursor (premalignant) lesions. In addition, keratoacanthoma (KA) which has to be viewed as a malignant cutaneous neoplasm by morphology is in almost all instances benign by behaviour. Because of their distinct genetic aberrations and the ability to spontaneously regress also KAs could be viewed as "precursors" to SCC (Burnworth et al., 2006 and for review see Boukamp, 2005a, b).

As proposed by Hanahan and Weinberg (2000), a number of molecular regulatory changes are required to make a tumour cell. These include a modified cell cycle control, onset of genomic instability, establishment of a telomere maintenance mechanism, and induction of angiogenesis. Depending on the tumour type, these changes can be brought about by different routes, and at different stages of tumour development. In SCC of the skin, only some of the responsible aberrations have been identified so far. The most obvious one is the p53 tumour suppressor gene mutational inactivation which is caused by UV-B radiation and which leads to genomic instability (reviewed in Duensing and Duensing, 2005). Further, the telomere length maintenance mechanism in skin keratinocytes is well established. But unlike in other tissues, in normal human skin keratinocytes the ribonucleoprotein complex telomerase is expressed (Harle-Bachor and Boukamp, 1996) and actively contributes to only a minor telomere loss with age (Moshir et al., manuscript in revision). Thus, telomerase activation in skin SCCs does not need to occur de novo but may only require an altered regulation. Concerning cell cycle deregulation, cyclin D1 has become a promising candidate for skin carcinogenesis (Utikal et al., 2005; Burnworth et al., 2006). We have shown recently that overexpression of cyclin D1 in the human HaCaT skin keratinocyte line resulted in a slight increase in proliferation, and most prominently, in a state of disturbed tissue homeostasis. Increased levels of cyclin D1 disturbed the generally tightly regulated balance between proliferation and differentiation, giving rise to highly disorganised epithelia (Burnworth et al., 2006). Since this chain of events did not directly lead to tumourigenicity, and as cyclin D1 overexpression was equally prominent in KAs and SCCs, cyclin D1 overexpression is probably an early change contributing to skin cancer development rather than progression.

However, less is known about changes adding to later stages of skin carcinogenesis. In a skin carcinoma model, we have shown previously that the matrix glycoprotein thrombospondin 1 (TSP-1), long known for its anti-angiogenic function (Good et al., 1990), can modulate the tumour phenotype. Overexpression of TSP-1 in the SCL-I skin carcinoma cell line (Boukamp et al., 1982) resulted in the reversion of the malignant phenotype by means of deposition of TSP-1 into the matrix at the tumour–stroma border with a concomitant, complete arrest of tumour vascularisation (Bleuel et al., 1999). The causal role of TSP-1 in the altered angiogenic and invasive response was verified by antisense-dependent inhibition of expression in the same tumour cell line: as expected, highly vascularised and locally invasive SCCs were obtained when TSP-1 was repressed (Bleuel et al., 1999).

Aiming to further unravel the role of such diverse aberrations in skin cancer development and progression,