Carcinogenesis vol.26 no.10 pp.1657–1667, 2005
doi:10.1093/carcin/bgi123
Advance Access publication May 19, 2005

COMMENTARY

Non-melanoma skin cancer: what drives tumor development and progression?

Petra Boukamp

Division of Genetics of Skin Carcinogenesis, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Email: P.Boukamp@DKFZ-Heidelberg.de

Non-melanoma skin cancer, i.e. basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most frequent tumors and their number is still increasing worldwide (1). Furthermore, immunosuppression in organ transplant patients strongly contributes to the increase in skin cancer incidence—being 65–250 times more frequent than in the general population. Often these patients suffer from a second and third lesion and the severity of these tumors is linked to their number. SCCs in transplant recipients also appear to be more aggressive. They tend to grow rapidly, show a higher rate of local recurrences and metastasize in 5–8% of the patients (all reviewed in Ref. 2). This largely differs from BCCs which are more frequent in the general population—at a ratio of 4:1 as compared with SCCs—but the number is only increased by a factor of 10 in transplant recipients. This may suggest that 'dormant' SCC precursor cells/lesions are present at a high frequency in the population but they are well controlled by the immune system. BCC, on the other hand, may be less dependent on immune surveillance thereby underlining its different etiology. While for BCC development the genetic hallmark is abrogation of the ptch-sonic hedgehog pathway, little is known about the causal alterations of SCCs. However, the complexity of the genetic alterations (numerical and structural aberration profiles) in SCCs argues for several levels of genomic instability involved in the generation and progression of skin cancer.

The multi-stage model of skin carcinogenesis (SCC development)

While BCCs are believed to develop *de novo*, skin SCC development is viewed as a multistep process. In agreement with that, cumulative life time exposure to ultraviolet (UV) radiation (especially UV-B) is thought to be the primary cause of skin carcinogenesis. Early and potentially primary event mutations of p53 have been found in otherwise unsuspicious epidermis (3–5) and can be detected as p53 patches by immunohistochemistry. Indeed, 70% of these p53 patches were shown to have an underlying mutation in the *p53* gene (4,6). It thus was reasoned that those are early precancerous lesions and may even serve as a risk marker for skin SCC development. However, a retrospective study of 250 cases did not establish statistically significant differences between skin from patients with solitary versus multiple skin carcinomas. Instead, this study highlighted an increased frequency of p53 patches with age (7).

Actinic keratoses (AK), on the other hand, are well established precancerous skin lesions and it is suggested that ~10% of these sun-induced lesions will develop into skin SCCs (8). With reference to this it is not surprising that p53 mutations and in particular UV-type mutations are frequently found in AK. Bowen's disease (BD), also known as carcinoma *in situ* (CIS), represents a preinvasive stage of the invasive skin SCCs. AK and CIS can occur at multiple sites as well as in the vicinity of SCC, supporting the hypothesis of field cancerization already suggested from the immuno-positive p53 distribution (9). Further indications for their precursor state came from molecular and cytogenetic studies (10–12). In contrast, a Japanese study that investigated microsatellite instability (MSI) and loss of heterogeneity (LOH) in AK and SCC showed no MSI in neither AK nor SCC and LOH in only 7/37 AKs and 1/14 SCCs. Since LOH was also demonstrated in histologically normal skin they suggested that at least in Japan AK was not likely to precede SCC (13).

Another skin tumor that is still controversially discussed in the sequence of skin carcinogenesis is the keratoacanthoma (KA). KAs are benign cutaneous squamous neoplasias arising preferentially on sun-exposed skin. They are characterized by a rapid growth phase for the first 4–8 weeks and a possible spontaneous self-induced regression after 3–6 months. Due to the initially very similar growth rate and morphology of KAs and well differentiated SCCs (14), it is still a matter of debate whether KAs are a specific type of SCCs or are not within the same biological and diagnostical spectrum (15). Several architectural criteria were used to distinguish KAs from SCCs. However, even in combination, these were not satisfying (16). While a previous LOH study did not support the hypothesis that KAs are SCCs that regress as a result of external (host) influences (17) our present data argue in favour of KAs being genetically incomplete SCCs and therefore are likely still controlled environmentally (S. Popp, B. Jelinek and P. Boukamp, unpublished data). Thus, it remains to be seen whether KAs may still best be viewed as an aborted malignancy that only rarely progress into an invasive SCC as proposed by Schwartz (18) or are in most cases genetically incomplete versions of SCCs.

Abrogation of UV-damage response

A role for the p53 tumor suppressor gene

Which are the genetic lesions that characterize skin carcinoma development and progression? The most prominent and the

Abbreviations: AK, actinic keratoses; BCC, basal cell carcinoma; BD, Bowen's disease; BFB, bridge-fusion-breakage model; CIS, carcinoma *in situ*; DMBA, di-methyl-benzanthracene; ds, double strand; HPV, human papillomavirus; KA, keratoacanthoma; LOH, loss of heterogeneity; LOHZ, loss of heterozygosity; MCC, Merkel cell carcinomas; M-FISH, multiplex fluorescence *in situ* hybridization; MSI, microsatellite instability; NBCC, nevoid basal cell carcinoma syndrome; SCC, squamous cell carcinoma; SHH, sonic hedgehog; TAs, telomeric aggregates; UV, ultraviolet.

best studied aberration in skin cancers is the mutation of the p53 tumor suppressor gene. While early studies suggested as many as >90% of SCCs and >50% of BCCs being mutated in the *p53* gene (19), it is now well established that ~50% of all skin cancers show mutations and this frequency rises to 90% in skin cancers with xeroderma pigmentosum, a recessive disorder associated with a defect in nucleotide excision repair that predispose to skin cancer (reviewed in Ref. 20). Most importantly, many p53 mutations are C to T transitions with a high frequency of CC to TT double base changes, thus being indicative of UV-radiation-induced mutations (21,22). For example, in colon cancer the second p53 allele is often lost during tumor progression (23), whereas it is intriguing to note that in skin carcinomas both the alleles can be mutated, with each allele carrying a different and mostly UV-type specific mutation (19,24). In addition, the mutations do not appear to be at random but show a pattern of hot spots different from that of internal malignancies (25). Giglia-Mari and Sarasin (20) recently proposed that from these mutational hot spots mutations in codon 177 are specific only for BCCs while mutations in codon 278, although mutated at a lower frequency also in certain internal cancers, seem to be specific for skin SCCs. Furthermore, since evidence is increasing that certain mutations may not only abrogate p53 function but may represent gain of function mutations (reviewed in Ref. 26), understanding the functional consequence of these specific p53 mutations still remains an important goal for a better understanding of the mechanisms involved in skin cancer development.

A role for the human papillomavirus (HPV)

Similar as by mutations, p53 function is also abrogated in human skin carcinomas by the HPV the action of which was extensively reviewed recently by Duensing and Münger (27). More than 100 subtypes have been identified but only a subgroup of so called high-risk mucosotropic HPV types, including HPV-16, 18, 31, 33, 35 and 58, are believed to be a causative agent in the development of cervical cancer. The *E6* gene of these high risk HPV types is able to induce rapid proteasomal degradation of p53 thereby abolishing the induction of cell cycle arrest or apoptosis. As in cervical carcinomas, HPV DNA is also frequently detected in skin carcinomas. A high prevalence of HPV infection seems to be characteristic for immunocompetent (47%) and even more for immunosuppressed patients (75%). However, >40 different HPV types were identified (28) and the typical high risk HPV types (e.g. HPV 16 and 18) were not yet found in skin carcinomas (reviewed in Ref. 29). Therefore, it was suggested that mechanisms different from the activity of HPV oncoproteins in genital cancer may be involved in skin neoplastic transformation (30).

Recently it was shown that HPV38, which was detected in ~50% of skin carcinomas but only in 10% of healthy skin, was able to actively support longevity/immortalization of cultured human skin keratinocytes (31). An Australian study further demonstrated HPV-38 DNA in 43% of solar keratoses, as well as 13 and 16% of SCCs and BCCs, respectively (32). In addition, HPV-8, thought to be involved in the rare inherited disorder of epidermodysplasia verruciformis that is characterized by the life-long occurrence of multiple flat warts and macular lesions, was expressed in the epidermis of mice (Keratin 14 promoter) and these HPV-8 transgenic mice spontaneously developed single or multiple benign skin tumors (33). Interestingly, HPV-16 transgenic mice (also under the K14 promoter) only developed hyperplasia associated with hyperkeratosis (34). All these still controversial findings together with the high frequency of HPV DNA in normal skin—particularly in hair follicles—and the fact that many different and often several HPV types are found concomitantly in normal and tumor tissues makes their role in skin carcinogenesis still elusive. Furthermore, a recent report that HPV DNA was frequently detected in swap samples collected from the superficial layers of skin tumors while only little HPV DNA was found in biopsies of the same tumors (35) strongly substantiates a rather critical attitude.

A role for cell cycle inhibitors

One cell cycle inhibitor that plays an important role in epithelial transformation is the cyclin-dependent kinase inhibitor $p16^{INK4}$ that specifically inhibits progression through G1 phase of the cell cycle by blocking the cyclin-dependent kinase 4 from phosphorylating the retinoblastoma protein (36). The INK4a locus encodes another structurally and functionally independent protein, $p14^{ARF}$, which is also believed to be a potent tumor suppressor (reviewed in Ref. 37). $p14^{ARF}$ activates the p53 pathway in response to oncogenic signals, such as the *c-myc* or *ras* oncogene, by binding to the p53 negative regulator Mdm2 and preventing p53 degradation thereby inducing cell cycle arrest or apoptosis. Since p53 is often mutated in skin carcinomas and is likely to be an early event, elimination of $p16^{INK4}$ rather than $p14^{ARF}$ seems to be involved in skin cancer development. Accordingly, $p16^{INK4}$ mutations were detected, although at a low frequency, in the general population and also in patients suffering from xeroderma pigmentosum (38–40), whereas $p14^{ARF}$ mutations were not yet reported. It was discussed that $p16^{INK4}$ mutations may be late events in skin cancer development and therefore may have been missed in studies evaluating precancerous lesions or small tumors, i.e. microinvasive SCCs (40). On the other hand, loss of heterozygosity (LOH) as well as loss of parts or the entire short arm of chromosome 9—$p16^{INK4}$ maps to 9p21—are frequently observed in SCCs (24,41). This raises the question whether in skin carcinomas loss of the $p16^{INK4}$ function is rather a result of loss of one copy of chromosome 9p and silencing of the second copy by epigenetic mechanisms such as methylation. However, it also cannot be excluded entirely that inactivation of $p16^{INK4}$ may be less essential and another gene on chromosome 9p, which still needs to be identified, is crucial for skin cancer development.

Even though inhibition should lead to the absence of $p16^{INK4}$ protein, dysplastic and neoplastic epithelial cells from the cervix uteri were shown to frequently upregulate $p16^{INK4}$ as determined by immunostaining (42). Since cervical cancer is closely associated with HPV infection it is tempting to speculate that this upregulation may be causally related to HPV. Presently, two immunohistochemistry studies, evaluating $p16^{INK4}$ expression in AK, CIS (Bowen's disease), and SCCs of the skin, presented rather controversial results. Hodges *et al.* (43) found immunostaining in about all AKs and *in situ* SCCs but only in 30% of the invasive SCCs. Owing to an increase in staining intensity in AK to invasive SCC, they nevertheless proposed a positive correlation between expression of $p16^{INK4}$ and skin cancer progression. Salama and co-workers showed a high frequency of immunostaining for BD but very little for AK and none for SCC. From this, they concluded that $p16^{INK4}$ is a selective and specific marker to distinguish BD from KA/benign SCC (44). Our data further add to this

confusion. We found $p16^{INK4}$ immunostaining in about all KAs but only few SCCs (B. Jelinek *et al*., manuscript in preparation). Interestingly, all $p16^{INK4}$ positive SCCs were poorly differentiated. This may allow two interpretations. First, $p16^{INK4}$ expression is required for KAs to control their growth and thus allow tumor regression. Second, the differential expression of $p16^{INK4}$ in well versus poorly differentiated SCCs may highlight two different developmental pathways. Thus, contributions of the cell cycle inhibitor CDKNA2/$p16^{INK4a}$ to skin cancer development is still far from being understood.

Alternatively to changes in 16^{INK4}, abrogation of 14-3-3sigma, a member of a protein family that regulates cellular activity by binding and sequestering, e.g. cyclin B1 and cdc2, may substitute for an aberrant growth control in skin tumor cells. 14-3-3-sigma, which is controlled by p53, promotes premitotic cell cycle arrest following DNA damage and thereby prevents 'mitotic catastrophy' (45). Even though expressed at high levels in AKs and skin SCCs, 14-3-3sigma expression was partially or completely lost in BCCs mainly due to CpG-hypermethylation (46). It is generally believed that $p16^{INK4}$ remains wildtype in BCCs and accordingly (no methylation was found in the above study) it was suggested that 14-3-3sigma may substitute for $p16^{INK4}$ in BCCs, and the silencing of 14-3-3sigma may thus contribute to the evasion of senescence in BCC (46). Different from BCCs, abnormal 14-3-3sigma expression is not yet described for SCCs. Thus, further studies need to clarify whether or not a preference for 14-3-3sigma or $p16^{INK4}$ may be tumor (BCC versus SCC) type-specific.

Contribution of the ras oncogene

Another gene that is likely to be mutated by the UV radiation but is controversially discussed concerning its contribution to skin cancer development is the *ras* oncogene. From the three *ras* genes, *Harvey*-(*Ha*), *Kirsten*-(*Ki*) and *N-ras*, mutations in *Ha-ras* predominate in the general population with the mutations characteristically seen at codons 12, 13 and 61—all localized opposing UV-sensitive CC sites. Mutation frequencies were extensively analyzed in the early 1990s and at that time it was proposed that the *ras* oncogene significantly contributes to skin cancer development. Now, an overall mutation frequency of 10–20% is suggested for SCCs and BCCs (24,47–52). On the other hand, a high mutation frequency and prevalence of *N-ras* mutations was reported for xeroderma pigmentosum patients being consistent with an even more important role of UV-induced lesions and a different mutation profile in these patients (53).

This latter situation may be reflected by the best established and most frequently used mouse model for skin carcinogenesis, the initiation–promotion protocol. Using one treatment of the carcinogen di-methyl-benzanthracene (DMBA) followed by twice weekly application of the phorbole ester (TPA) causes a ras-dependent formation of papillomas that in part progress to SCCs (reviewed in Ref. 54). Although the tumors formed in this model show remarkable phenotypic similarities with human skin carcinogenesis, how far the molecular findings based on chemical carcinogenesis and on 'ras-dependent' skin carcinogenesis can be extrapolated is still an open question. The answers will help to unravel the as yet still poorly understood genetic pathway involved in human UV-induced skin carcinogenesis. For this, the SKH1 hairless mouse may represent a more relevant model (for a review see Ref. 55). With these mice, reproducible and quantitative data could be established on dose, time and wavelength of the UV radiation required for the SCC development (56). A high frequency of p53 mutations were found, whereas only 1 out of 32 tumors carried a *ras* mutation (57). Furthermore, the majority of the p53 mutations were located in a codon matching with a hotspot for p53 mutations in human skin carcinomas, thus largely mimicking the essentials of human UV-induced skin cancer. However, careful consideration is required when extrapolating results from these mice to humans. Differences exist, e.g. in the composition of melanin and with that in the protection against UV-radiation. Increased pheomelanin photosensitizes DNA and thus induces DNA damage. Depending on the skin type, various compositions of pheomelanin and an another form of melanin, the eumelanin, are observed. Eumelanin predominates in mouse melanocytes (58).

Contribution of the patched tumor suppressor gene

While the functional contribution of the above aberrations are still far from being understood, one aberration—loss of 9q—originally detected in the autosomal dominant disorder termed Gorlin or nevoid basal cell carcinoma (NBCC) syndrome (59) has significantly advanced our understanding of the origin of BCCs. NBCC syndrome, which is characterized by multiple BCCs at an early age (60,61), not only allowed indentification of the putative tumor suppressor gene, the *PTCH* gene (62,63), but also led to the identification of an important pathway, the hedgehog-patched-smoothened pathway, which is abrogated in >70% of BCCs.

In skin, sonic hedgehog (SHH) signaling has been implicated in hair follicle growth and morphogenesis. Patched1, the protein product of *PTCH*, is a cell surface receptor of the secreted signaling molecule SHH. In the absence of SHH, patched1 inhibits smoothened (SMO), a G-protein-coupled-like receptor. SMO is released upon binding of SHH to patched1 and can initiate a signal transduction cascade that causes activation of the transcription factor Gli1. Thus, dysregulation of this pathway by either the loss of *PTCH* or the forced expression of *SMO* results in elevated levels of the transcription factor Gli1 and, as a consequence, induces hair follicle tumors (64–67) by opposing cell cycle arrest and differentiation (68). Mutations of either *PTCH* or *SMO* have been found in >70% of sporadic human BCCs (69,70). Because of this high correlation it is generally accepted that abrogation of this pathway is the major cause of BCC development and that BCCs are hair follicle-derived tumors (71). Furthermore, Louro *et al*. recently performed a comparative gene expression profiling and suggested that Gli1-induced transcripts may convert normal keratinocytes into invasive tumor cells in a relatively direct fashion, without the need of other multiple genetic aberrations (72).

Accordingly, also mouse models demonstrated that abrogation of the ptch-sonic/hedgehog pathway (Ptch ± mice) is essential for basal cell tumorigenesis (65) and that overexpression of Gli1, the zink-finger transcription factor activated by hedgehog, is central and probably sufficient for tumor development (66). Interestingly, in mice, the over expression of Gli1 under the epithelial keratin 5 (K5) promoter developed predominantly follicle-derived tumors and only a few BCCs (66). However, K5-Gli2 transgenic mice exclusively developed BCCs (67). Thus, it will be of interest to determine whether a similar distribution will be seen in human tumors.

Also in SCCs, the chromosomal region 9q22 to q31 is frequently involved in aberrations. Some tumor cells show gain of the entire q-arm due to the formation of an iso-chromosome i(9q) [(24) and unpublished data], whereas LOH in 9q22.3 was reported to be characteristic for carcinomas *in situ* and SCCs, indicating that these alterations are late events in the SCC development (73). Although it was suggested that interfollicular epidermal cells can express hair follicle markers when exposed to powerful morphogens such as *SHH* and *WNTs* (74), there is no indication to date that *patched* plays a role in SCC development. Even more, while BCCs are generally associated with hyperactivation of the sonic hedgehog signaling pathway causing a continuous activation of this growth promoting cascade (reviewed in Ref. 75), evidence for a specific signal transduction pathway forcing SCC development is still missing.

Molecular and cytogenetic changes

What is the difference between SCCs and BCCs that no specific developmental pathway could yet be established for SCCs? Which other aberrations could account for SCC development? Already in 1994, Rees and coworkers reported on LOH of 9q in as many as 77% of BCCs but only in 12% of SCCs. In contrast, LOH of 9p markers was frequent in SCC (41). In addition, a number of other chromosomes were shown to carry LOH in SCCs such as 3p, 13p, 17p and 17q (10). Comparison between AKs and KAs revealed that AKs shared many of the same loci as SCCs, whereas the frequency of LOH in KAs was low with only isolated losses at 9p, 9q and 10q (10,17).

By cytogenetic analysis and comparative genomic hybridization, which allows a genome wide screen of gains and losses (76), SCCs demonstrated a great karyotypic complexity and cytogenetic heterogeneity, whereas the aberration profile is much simpler in AKs and KAs (12,24,77,78). Jin and colleagues found that in SCCs many structural aberrations affecting centromeric regions, particularly those of chromosomes 3q, 8q, 9q and 5p, leads to whole-arm translocations and the duplication of one chromosome arm thereby causing the formation of iso-chromosomes (12). Additionally, they observed genetically unrelated clones within the same tumor, suggesting that a multi-focal development is rather frequent in skin cancer. AKs, on the other hand, only revealed few numerical changes, e.g. gain of chromosomes 7, 9 and 18, that were common, while recurrent iso-chromosomes were not seen (12). Our CGH studies suggest that gains and losses of the same chromosomes (in particular loss of 3p and 9p, and gain of 11q) are involved in KAs and SCCs. The important difference, however, is that the individual KA only carries one or two of these aberrations while they are commonly present as a combination in SCCs (S. Popp, B. Jelinek and P. Boukamp, unpublished). This further strengthens the hypothesis that, as discussed above, KAs may be genetically incomplete SCCs. Finally, multiplex fluorescence *in situ* hybridization (M-FISH), which can identify all chromosomes of a metaphase simultaneously (79), highlighted complex translocations in the SCC derived cell lines (78) substantiating the important role of genomic instability in the skin SCC development.

So far, the reason for the high frequency of chromosomal aberrations such as deletions, amplifications and translocations, causing the substantially deranged karyotypes of SCCs, is still elusive. Since BCCs (80) as well as Merkel cell carcinomas (MCC) (24,81), which are highly aggressive endocrine tumors of the skin that develop on the same sun exposed sites, exhibit significantly less chromosomal changes per tumor, the complex karyotype of SCCs cannot simply be attributed to the environment and to the recurrent damage by the UV radiation. It also does not correlate with tumor aggressiveness, because MCCs show a much higher frequency of recurrences and metastases than SCCs (82). However, the large spectrum of aberrations in SCCs and precursor lesions may provide a broad basis for the potentially tumorigenic cells that can more rapidly adapt to environmental changes, and thus may explain the high frequency of SCCs occurring in immune-suppressed patients after a relatively short latency period.

Human skin cancer progression models

Different from the initiation promotion skin cancer mouse model, two human model systems have been described that show the typical SCC aberration pattern and therefore are well suited to elucidate functional consequences of the above described genetic aberrations. The MET model consists of cell lines derived from a primary tumor, two recurrences, and a metastasis from the same patient (83). Genetic analysis of tumors and their derived cell lines provided evidence that the metastasis directly evolved from the primary tumor while the two recurrent tumors had developed independently from a precursor lesion. Furthermore, these studies demonstrated that the tumors and derived cell lines were genetically highly comparable and carried a number of aberrations typically seen in SCCs (78). Interestingly, these cells are devoid of p53 mutations and HPV-sequences (83) thus making this sequence of human SCC cells a unique *in vivo* progression model to study p53-independent changes in skin carcinogenesis.

Another model, frequently used for functional studies in human skin carcinogenesis, is based on the spontaneously immortalized HaCaT cells (84). In contrast to the MET cells, the HaCaT cells exhibit UV-type specific mutations in both alleles of the *p53* gene (85). In addition, they carry chromosomal aberrations characteristically seen in SCCs (86). Introduction of the *Harvey-ras* oncogene (codon 12 mutation) allowed to establish benign and malignant tumorigenic variants (87,88), and *in vivo* selection finally led to metastatic conversion (89). With this sequence of different stages of skin carcinogenesis, the HaCaT cells already served as a suitable model to study the functional consequences of the overexpression and suppression of a number of genes associated with skin cancer progression (88,90–94).

Genomic instability as a driving force in skin cancer development

Studies using the HaCaT cells have shown that mutational inactivation of p53 and some other genetic aberrations typically found in skin carcinomas (loss of e3p, gain of 3q and loss of 9p—one copy each) were not sufficient to render the cells tumorigenic. Accordingly, tumorigenic conversion did not occur spontaneously during long-term propagation *in vitro* and this correlated with a rather stable genotype (86). However, exposing the cells to an increased temperature and thereby inducing an oxidative damage response caused formation of new chromosomal aberrations and tumorigenic

conversion (90). Similarly, introduction of the ras oncogene *per se* did not induce tumor formation but required the cooperation of genetic changes that manifested in a step-wise fashion during the long-term propagation (87,88). In good agreement with that, the most consistent difference between precancerous skin lesions and SCCs is the increase in karyotypic complexity. This suggests that destabilizing the genome is an important driving force in skin cancer progression. Furthermore, there is increasing evidence that more and more factors are contributing to genomic instability and this provides the possibility for ever-new phenotypes including metastatic and drug-resistant ones (95).

How can p53 contribute to the accumulation of further genetic changes?

As discussed above, mutations in the *p53* gene are believed to be a very early if not initial event in skin carcinogenesis (21). As a guardian of the genome (96), p53 is stabilized upon stress by phosphorylation and alters the expression of different sets of downstream target genes including those that cause a cell cycle arrest (97). Thus, the resulting damage, e.g. double strand (ds) breaks, can be repaired thereby preventing gross chromosomal changes including amplifications and deletions. In the p53 mutant cells, cell cycle arrest is abolished. This may allow, as a first step of genome destabilization, non-lethal chromosomal damage to be passed on to the daughter cells. Such a scenario is suggested in the case of human tumor cells that show a good correlation between mutation in the *p53* gene and a genome wide instability as evidenced for colorectal cancers by karyotypic abnormalities, chromosomal amplifications and genome-wide allelic imbalances (98–100). Furthermore, Overholzer and colleagues recently showed that mutations in the *p53* gene but not amplified HDM2, which inhibits the tumor suppressive properties of p53 by controlling p53 degradation, correlated with high levels of genomic instability in osteosarcomas (101). Therefore, they suggested a qualitative difference for direct mutational versus indirect HDM2-dependent inactivation in destabilizing the genome. On the other hand, Carroll *et al*. (102) provided evidence that p53 mutations as well as MDM2 overexpression, induced aneuploidy through centrosome amplification. Centrosome duplication, which has to occur with each cell cycle, is thought to be controlled by the phosphorylation status of the retinoblastoma protein, release of the E2F transcription factor and subsequent transcriptional activation of the cyclin-dependent kinase 2 late in G1 (reviewed in Ref. 103). From this it was proposed that the frequent abrogation of the Rb pathway may not only facilitate progression towards DNA replication but may also deregulate the centrosome duplication cycle (104) generating abnormal chromosome segregation and concomitantly aneuploidy.

Centrosome-mediated defects were also suggested for HPV-induced genomic instability (reviewed in Ref. 27). It is long known that HPV-16 E6 can cause structural chromosomal changes (105), whereas E7 is correlated with numerical chromosomal abnormalities resulting in aneuploidy (105,106). Duensing and colleagues further showed that E6 and E7 cooperate to induce genomic instability by uncoupling centrosome duplication from cell division (107) and that E7 is responsible for abnormal centrosome synthesis (108). In skin carcinomas, mutations in the *p53* gene as well as HPV DNA sequences are frequently detected. It thus remains to be seen whether one or both may be responsible for the complex aberration profile by the initiation of genomic instability through deregulation of the centrosome duplication cycle.

Telomere dysfunction

Recently, a strong association of chromosomal rearrangements and telomere dysfunctions was proposed—thus referring to the original findings by Hermann Muller and Barbara McClintock who demonstrated in the 1930s that the ends of the chromosomes, the telomeres, play a crucial role in maintaining chromosomal stability. McClintock showed that following the breakage and fusion of maize chromosomes, loss of the ends of the chromosomes rendered the chromosomes highly recombinogenic (109).

As known today, these observations long preceded the genetic definition of a telomere that consists of thousands of repeats of the hexanucleotid TTAGGG and makes up to 15 kb in human cells. The double stranded telomeric DNA ends in a 3′ single stranded overhang that is believed to be required for a higher order structure (reviewed in Ref. 110). One important model is that the telomeres form loop structures, t-loops, and by invasion of the 3′ overhang into the duplex region of the double stranded telomeric DNA, protect the DNA against degradation and end-to-end fusion, thereby providing a protective cap for the chromosomes (111). These caps are, however, 'finite' owing to the failure of the DNA polymerase α to replicate linear DNA to the outermost end (112). This so-called end-replication problem causes a replication-dependent telomere loss, which in many normal somatic cells is manifested as a pattern of continuous telomere erosion and is now assumed to be the counting mechanism, the endogenous clock of cellular aging (113). In germ line and tumor cells a specialized ribonucleoprotein complex, telomerase, is expressed that can prevent telomere shortening by *de novo* synthesis of telomeric sequences and thus can maintain the capping function of the telomeres (110).

Studies with telomerase-deficient mice have shown that in late generations end-to-end chromosomal fusions were the most prominent aberrations (114). Since they frequently involved the chromosomes with the shortest telomeres, critically short and therefore unprotected telomeres were thought to be a primary cause of these end-to-end fusions (115). Similarly, uncapping of the telomeres by overexpressing a dominant-negative version of the telomere repeat binding factor TRF2 further substantiated that in human cells also uncapping of the telomeres resulted in chromosomal end-to-end fusions thereby generating dicentric chromosomes (116). If both the centromeres are active, dicentric chromosomes are highly unstable during mitosis. If each centromere is attached to a different pole these chromosomes form anaphase bridges, a situation commonly viewed as a marker for genomic instability. As a result of this bipolar tension, breakage can occur anywhere along the chromosome. This led to the proposal of a fusion-bridge-breakage cycle as a primary mechanism of genomic instability in cells that suffer from critically short telomeres (117). The highly recombinant telomere-free DNA ends may invade other chromosomes, perhaps based on areas of micro-homology, thereby yielding non-reciprocal translocations. This hypothesis, also known as Bridge-fusion-breakage model (BFB), is now extensively used to explain the various translocation chromosomes found in tumor cells (for a review see Ref. 118).

Alternatively, Greider and co-workers (119) suggest that end-to-end chromosome fusions may not initiate

rearrangements but may rather be a secondary effect of end resection and thus represent stable byproducts. From the study of chromosomal rearrangements in diploid yeast strains, they found that chromosomal rearrangements predominantly occur in the terminal region of the chromosome. Since exonuclease Exo1p played a major role in the generation of these rearrangements, they suggested that end resection initiates genomic instability at dysfunctional telomeres.

Trying to unravel factors responsible for impaired telomere integrity, the telomerase-deficient mice were crossed with mice deficient in a number of genes implicated in the maintenance of the telomeres such as the repair proteins Ku86, the subunit of DNA-PK, DNA-PKcs and the poly(ADP)ribosyl polymerase PARP-1 (120,121). While it was shown that PARP-1 had no effect in telomere metabolism and therefore did not cause obvious changes, Ku86 and DNA-PKcs-deficiency caused accelerated loss of viability. This correlated with proliferative defects and age-related pathologies, but did not lead to an increased cancer incidence (122).

This may be unexpected because late generation telomerase knockout mice were shown to suffer from an increased rate of spontaneous tumors (123). Although it was proposed that the majority of tumor types originated from highly proliferative cell types, i.e. cells that were likely to sustain the highest degree of telomere shortening with increased age, the number of spontaneous skin carcinomas did not increase significantly. Gonzales-Suarez *et al*. (124) even reported on a dramatic inhibitory impact on chemical skin carcinogenesis (DMBA/TPA treatment) in the telomerase knockout mice providing evidence that short telomeres in the absence of telomerase activity could suppress tumor initiation. Along that line, absence of telomerase in transgenic mice expressing HPV16 had little effect. Genetic analyses of tumors from these mice did not provide evidence for telomere dysfunction or increased genomic instability (125).

Constitutive overexpression of telomerase (mTERT transgenic mice), on the other hand, promoted mammary carcinomas (126). While a skin cancer phenotype was not reported for these animals, transgenic mice over-expressing mTERT in an epithelia-specific manner—under the K5 promoter—revealed an increased number in papillomas when subjected to the DMBA/TPA protocol suggesting an increased tumor susceptibility upon telomerase activation. Interestingly, the conversion rate to carcinomas was not augmented (127). These mice also developed spontaneous tumors in the lung, mammary glands and the uterus with lymphomas being the most frequent tumor type (128). Stratified epithelia only showed hyperplasia and hyperkeratosis but no tumors arguing for telomerase up-regulation to be an early event in skin carcinogenesis and being related to proliferation rather than tumorigenic conversion. mTERT transgenic mice crossed into a p53 null background showed a similar tumor spectrum, though with a generally higher tumor yield (128). Since p53 mutations are frequent in skin cancers and as discussed above represent quite a specific mutation spectrum, it remains to be elucidated how far these mutations provide gain of function (26) and accordingly, whether abrogation of p53 can replace p53 mutations in order to elucidate the mechanism of human skin cancer development and progression.

Taken together, these findings rather argue in favour of telomerase as a driving force in tumor initiation than critically shortened telomeres being the basis of unstable and therefore susceptible chromosomes. If so, 'telomere length-independent' mechanisms of genomic instability are required.

Putative recombination models in telomerase-positive tumor cells

While uncapping of critically short telomeres predominantly should account for normal somatic and telomerase-negative cells and therefore would represent an initial stage in the transformation process, tumor cells generally express the telomerase and thus protect their telomere lengths. Nevertheless, there is ample evidence that these cells also suffer from genomic instability and that this is not a continuum of the telomere length-dependent BFB but can occur *de novo* upon induction of certain oncogenes e.g. the *c-myc* oncogene [for a review see Mai and Mushinski (129)].

Genomic instability induced by the c-myc oncogene

One recurrent chromosomal aberration in SCCs the is gain of chromosome 8q through the formation if an iso-chromosome. Since 8q harbors the *c-myc* gene, located at 8q24, *c-myc* expression should be increased. As a result of this aberration, *c-myc* amplification was described for 50% of SCCs from renal transplant patients (130). In addition to its role in proliferation, there is increasing evidence that one important consequence of deregulated *c-myc* expression is the induction of genomic instability. c-Myc seems to favor gene amplification and gene rearrangements as well as karyotypic instability leading to numerical and structural chromosomal aberrations (for a review see Ref. 129). The most intriguing features of c-Myc-induced alterations are their reversibility. Using inducible systems, genomic instability proved to be transient upon a single induction of c-Myc *in vitro* and *in vivo* (131,132). Continued c-Myc activation, on the other hand, was described to be accompanied by numerical and structural chromosomal changes such as extra-chromosomal elements and chromosomal breakage but also centromere-telomere fusions. (133). Furthermore, in the absence of p53 the c-Myc effect seemed to be augmented (134) and aberrant centrosome duplication was discussed as a potential mechanism.

c-Myc may additionally contribute to genomic instability through a telomere organization-dependent mechanism. While in normal cells the telomeres are present as non-overlapping territories, tumor cells (*in vitro* and *in vivo*) with deregulated c-Myc as well as HaCaT cells constitutively expressing the *c-myc* oncogene, showed aggregation of telomeres in a high percentage of cells (135,136). Since these telomeric aggregates (TAs) could be detected in interphase nuclei as well as in mitotic figures and the percentage of apoptotic cells was not significantly increased as compared with the parental cells (136), their presence throughout the cell cycle is likely to contribute to unequal segregation of the chromosomes during mitosis, i.e. numerical aberrations. In addition, TAs also likely force the chromosomes to alter their location, thereby providing an explanation for how the different chromosomes come into such close vicinity that they are able to exchange chromosomal material and give rise to the in part very complex multi-chromosomal translocations characteristically seen in skin carcinoma cells (137).

Telomere-independent mechanisms of chromosomal instability

Fragile sites. Cuillo *et al*. provided convincing evidence that not only critically short telomeres initiate BFB. They showed that the *PIP* (prolactin-induced protein) gene which is

overexpressed in 60–80% of primary and metastatic breast cancers (138,139) was duplicated in a breast carcinoma cell line due to one cycle of BFB. In their model, the first break was initiated at the fragile site FRA7I telomeric to the *PIP* gene (140). They proposed that fusion occurs after replication of the two sister chromatids leading to anaphase bridges. Breakage now results in one deleted and one amplified daughter cell. Although the mechanism responsible for activation of fragile sites in cancer cells is not fully understood there is evidence that stress resulting from oxygen starvation (hypoxia) and subsequent variations in tumor microenvironment can activate some chromosomal fragile sites. It is also possible that fragile site expression may result from spontaneous changes in chromatin organization and/or impairment of the rate of timing of DNA replication (141).

FRA3B, the most active common fragile site in the genome is mapped to chromosome 3p14.2, a chromosomal location frequently deleted in skin carcinomas. Furthermore, FRA3B is encompassed by the *FHIT* gene (fragile histidine triad), a putative tumor suppressor that is lost in various tumors (reviewed in Ref. 142). However, expression studies from different skin tumors as well as skin cancer cell lines exhibiting loss of one copy of chromosome 3p, demonstrated normal transcripts of the *FITH* gene (24,143). This suggests that the *FITH* gene is not a common target for deletions and that FRA3B is not a common break point in skin carcinomas.

DNA double strand breaks. While fragile sites favor the specific sites for DNA breaks and rearrangements, DNA ds breaks induced either in a replication-dependent manner or through exogenous insults e.g. reactive oxygen species produced by ionizing radiation or UV-A radiation are likely to occur throughout the genome and thus should cause a more random breakage. Interestingly, Rief (144) recently found that repair of ds breaks, which is dependent on non-homologous end-joining, is also dependent on the chromatin state. They demonstrated that restriction fragment reconstitution was considerably more efficient in centromeres than in the non-centromeric locations. Since there is some experimental evidence that chromosomal exchange aberrations are less frequent in genomic regions with highly condensed heterochromatin (145–147), this finding may support the hypothesis that the chromatin state is an important determinant of whether breaks are forced to rejoin correctly or can undergo non-homologous end-joining with DNA from other parts of the chromosome or even other chromosomes. The authors further discuss that not only the primary structure—the high number of repetitive elements—may increase the probability for homologous repair mechanisms but also the degree of chromatin condensation may limit the mobility of the broken ends. This could additionally lead to a much higher probability of properly rejoined breaks than at an average genomic location. Thus it is tempting to speculate that the intra-genomic heterogeneity of chromatin condensation may be one potential explanation for the tissue-specific prevalence of certain chromosomal alterations.

Centromeric break. The most common aberrations in skin SCCs are the centromeric breaks either leading to non-reciprocal translocation of different chromosome arms or to loss of one chromosome arm and duplication of the other, resulting in the formation of an iso-chromosome. The mechanism by which these aberrations evolve is still elusive. Recently Barbouti *et al.* (148) mapped the breakpoint region

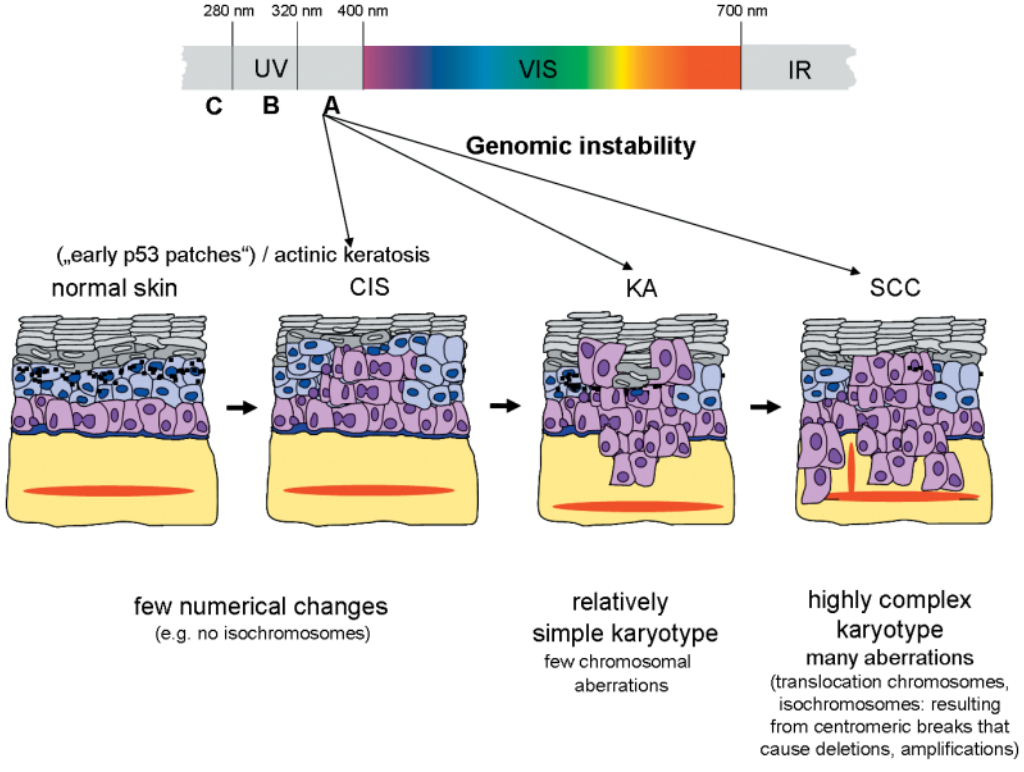

Fig. 1. Schematic representation of the potential developmental stages during UV-induced skin carcinogenesis. Both UV-B and UV-A can contribute to skin cancer at different developmental stages by directly inducing DNA damage (e.g. UV-B-dependent p53 mutations) or indirectly by UV-A-induced oxidative stress generating genomic instability. Genomic instability is likely to participate in early development to generate the first chromosomal changes (relatively simple karyotype) as well as during later stages (transition to a malignant SCC) to contribute to the highly complex karyotype.

of i(17q), the most common iso-chromosome in human hematopoietic malignancies, and found it to be dicentric. They identified large, palindromic and low-copy repeats, and proposed that breakage at these palindome structures allows reunion between palindromes on sister chromatids and the formation of both dicentric and acentric structures. While the acentric part is lost, the dicentric part is retained through mitoses without disruption. This is possible because the centromeres are in such close proximity that one of them is inactivated. The authors further concluded that such somatic rearrangements are not random events but rather reflect susceptibility owing to the genomic structure (148).

Centromeres are the primary site for kinetochore formation, the protein complex that binds to the spindle microtubules in order to coordinate chromosome segregation to the opposite poles during mitosis. A second important activity of the centromeres is the establishment and maintenance of cohesion (for a review see Ref. 149). Therefore as an alternative hypothesis, forced cohesion of the two sister chromatids at the centromeres could favor breakage of the less adhesive arms and maintenance of the two tightly fixed sister copies which then could reestablish as iso-chromosomes. The chromosomes involved in iso-chromosome formation seem to be tissue-specific and in skin carcinomas often involve chromosomes 3q, 8q and 9q. It is now essential to determine whether the genomic structure of the centromeric region of these particular chromosomes differ in keratinocytes versus other cell types and therefore are part of the non-random chromosomal changes in skin SCCs.

Conclusions and further perspective

Comparison of the two non-melanoma skin cancers, BCC and SCC, clearly demonstrate that despite the increased knowledge about the role of a number of oncogenes, tumor suppressor genes and signal transduction pathways, the genetic mechanisms causing tumors such as skin SCCs are still poorly understood. On the one hand, BCCs exhibit a relatively simple genotype, with only few aberrations and a high degree of independence of the immune system. With the identification of an aberrant sonic hedgehog pathway, a major cause of BCCs development was identified. Correspondingly, the plant-derived teratogen cyclopamine has been shown to reverse the effects of oncogenic mutations of *SMO* and *PTCH* (150). Its efficacy in the BCC treatment in humans remains to be seen and thus its application as a successful therapeutic strategy to eliminate this highly destructive and most common tumor type.

On the other hand, skin SCCs develop, at least in part, from precursor lesions and accumulate a highly complex genotype (Figure 1). While most of the genes causing tumor development and progression are still unknown as is a signal transduction pathway that can be causally related to SCC development, the most intriguing feature is the great number of chromosomal aberrations and the recurrent involvement of specific chromosomes in gains, losses and translocations, e.g. loss of 3p, loss of 9p and gain of 3q, 9q and 11q. With the increasing evidence that certain chromosomal aberrations and in particular certain combinations of aberrations are required for a fully malignant SCC, it is now of importance to unravel the mechanisms underlying the induction of these genetic alterations. Recent studies have shown that not only UV-B but also UV-A is involved in the UV-induced skin carcinogenesis (reviewed in Ref. 151). It now needs to be explored whether or not the oxidative stress generated by UV-A exposure may be a factor causing such chromosomal changes, i.e. inducing genomic instability, if so, how far (152). Thus, understanding and fighting genomic instability may be a promising future approach particularly for those tumors such as skin SCCs that suffer from numerous and highly complex aberrations.

Acknowledgements

The author wishes to thank Drs Norbert Fusenig and Margareta Müller for helpful comments and Angelika Lampe for her help in editing the manuscript. This work was in part supported by the, European Union (LSHC-CT-2004-502943), as well as the Deutsche Krebshilfe eV.

Conflict of Interest Statement: None declared.

References

1. Euvrard,S., Kanitakis,J. and Claudy,A. (2003) Skin cancers after organ transplantation. *N. Engl. J. Med.*, **348**, 1681–1691.
2. Freedberg,I.M., Eisen,A., Wolff,K., Austen,K.F., Goldspith,L., Katz,S. and Fitzpatrick,T. (1999) Fitzpatrick's Dermatology in General Medicine. McGraw-Hill Health Professional Division, New York.
3. Nakazawa,H., English,D., Randell,P.L., Nakazawa,K., Martel,N., Armstrong,B.K. and Yamasaki,H. (1994) UV and skin cancer: specific p53 gene mutation in normal skin as a biologically relevant exposure measurement. *Proc. Natl Acad. Sci. USA*, **91**, 360–364.
4. Jonason,A.S., Kunala,S., Price,G.J., Restifo,R.J., Spinelli,H.M., Persing,J.A., Leffell,D.J., Tarone,R.E. and Brash,D.E. (1996) Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc. Natl Acad. Sci. USA*, **93**, 14025–14029.
5. Ling,G., Persson,A., Berne,B., Uhlen,M., Lundeberg,J. and Ponten,F. (2001) Persistent p53 mutations in single cells from normal human skin. *Am. J. Pathol.*, **159**, 1247–1253.
6. Ren,Z.P., Ponten,F., Nister,M. and Ponten,J. (1996) Two distinct p53 immunohistochemical patterns in human squamous-cell skin cancer, precursors and normal epidermis. *Int. J. Cancer*, **69**, 174–179.
7. Le Pelletier,F., Soufir,N., de la,S.P., Janin,A. and Basset-Seguin,N. (2001) p53 patches are not increased in patients with multiple nonmelanoma skin cancers. *J. Invest. Dermatol.*, **117**, 1324–1325.
8. Johnson,T.M., Rowe,D.E., Nelson,B.R. and Swanson,N.A. (1992) Squamous cell carcinoma of the skin (excluding lip and oral mucosa). *J. Am. Acad. Dermatol.*, **26**, 467–484.
9. Kanjilal,S., Strom,S.S., Clayman,G.L., Weber,R.S., el Naggar,A.K., Kapur,V., Cummings,K.K., Hill,L.A., Spitz,M.R. and Kripke,M.L. (1995) p53 mutations in nonmelanoma skin cancer of the head and neck: molecular evidence for field cancerization. *Cancer Res.*, **55**, 3604–3609.
10. Rehman,I., Takata,M., Wu,Y.Y. and Rees,J.L. (1996) Genetic change in actinic keratoses. *Oncogene*, **12**, 2483–2490.
11. Quinn,A.G., Sikkink,S. and Rees,J.L. (1994) Basal cell carcinomas and squamous cell carcinomas of human skin show distinct patterns of chromosome loss. *Cancer Res.*, **54**, 4756–4759.
12. Jin,Y., Jin,C., Salemark,L., Wennerberg,J., Persson,B. and Jonsson,N. (2002) Clonal chromosome abnormalities in premalignant lesions of the skin. *Cancer Genet. Cytogenet.*, **136**, 48–52.
13. Kushida,Y., Miki,H. and Ohmori,M. (1999) Loss of heterozygosity in actinic keratosis, squamous cell carcinoma and sun-exposed normal-appearing skin in Japanese: difference between Japanese and Caucasians. *Cancer Lett.*, **140**, 169–175.
14. Billingsley,E.M., Davis,N. and Helm,K.F. (1999) Rapidly growing squamous cell carcinoma. *J. Cutan. Med. Surg.*, **3**, 193–197.
15. Hurt,M.A. (2004) Keratoacanthoma vs. squamous cell carcinoma in contrast with keratoacanthoma is squamous cell carcinoma. *J. Cutan. Pathol.*, **31**, 291–292.
16. Cribier,B., Asch,P. and Grosshans,E. (1999) Differentiating squamous cell carcinoma from keratoacanthoma using histopathological criteria. Is it possible? A study of 296 cases. *Dermatology*, **199**, 208–212.
17. Waring,A.J., Takata,M., Rehman,I. and Rees,J.L. (1996) Loss of heterozygosity analysis of keratoacanthoma reveals multiple differences from cutaneous squamous cell carcinoma. *Br. J. Cancer*, **73**, 649–653.
18. Schwartz,R.A. (1994) Keratoacanthoma. *J. Am. Acad. Dermatol.*, **30**, 1–19.

19. Brash,D.E., Ziegler,A., Jonason,A.S., Simon,J.A., Kunala,S. and Leffell,D.J. (1996) Sunlight and sunburn in human skin cancer: p53, apoptosis, and tumor promotion. *J. Investig. Dermatol. Symp. Proc.*, **1**, 136–142.
20. Giglia-Mari,G. and Sarasin,A. (2003) TP53 mutations in human skin cancers. *Hum. Mutat.*, **21**, 217–228.
21. Ziegler,A., Jonason,A.S., Leffell,D.J., Simon,J.A., Sharma,H.W., Kimmelman,J., Remington,L., Jacks,T. and Brash,D.E. (1994) Sunburn and p53 in the onset of skin cancer. *Nature*, **372**, 773–776.
22. Tornaletti,S. and Pfeifer,G.P. (1994) Slow repair of pyrimidine dimers at p53 mutation hotspots in skin cancer. *Science*, **263**, 1436–1438.
23. Fearon,E.R. and Vogelstein,B. (1990) A genetic model for colorectal tumorigenesis. *Cell*, **61**, 759–767.
24. Popp,S., Waltering,S., Herbst,C., Moll,I. and Boukamp,P. (2002) UV-B-type mutations and chromosomal imbalances indicate common pathways for the development of Merkel and skin squamous cell carcinomas. *Int. J. Cancer*, **99**, 352–360.
25. Ziegler,A., Leffell,D.J., Kunala,S., Sharma,H.W., Gailani,M., Simon,J.A., Halperin,A.J., Baden,H.P., Shapiro,P.E. and Bale,A.E. (1993) Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers. *Proc. Natl Acad. Sci. USA*, **90**, 4216–4220.
26. Deppert,W., Gohler,T., Koga,H. and Kim,E. (2000) Mutant p53: 'gain of function' through perturbation of nuclear structure and function? *J. Cell Biochem. Suppl.*, **Suppl. 35**, 115–122.
27. Duensing,S. and Münger,K. (2004) Mechanisms of genomic instability in human cancer: insights from studies with human papillomavirus oncoproteins. *Int. J. Cancer*, **109**, 157–162.
28. Meyer,T., Arndt,R., Nindl,I., Ulrich,C., Christophers,E. and Stockfleth,E. (2003) Association of human papillomavirus infections with cutaneous tumors in immunosuppressed patients. *Transpl. Int.*, **16**, 146–153.
29. Pfister,H. (2003) Chapter 8: human papillomavirus and skin cancer. *J. Natl Cancer Inst. Monogr.*, 52–56.
30. Meyer,T., Arndt,R., Christophers,E., Nindl,I. and Stockfleth,E. (2001) Importance of human papillomaviruses for the development of skin cancer. *Cancer Detect. Prev.*, **25**, 533–547.
31. Caldeira,S., Zehbe,I., Accardi,R., Malanchi,I., Dong,W., Giarre,M., de Villiers,E.M., Filotico,R., Boukamp,P. and Tommasino,M. (2003) The E6 and E7 proteins of the cutaneous human papillomavirus type 38 display transforming properties. *J. Virol.*, **77**, 2195–2206.
32. Forslund,O., Ly,H., Reid,C. and Higgins,G. (2003) A broad spectrum of human papillomavirus types is present in the skin of Australian patients with non-melanoma skin cancers and solar keratosis. *Br. J. Dermatol.*, **149**, 64–73.
33. Schaper,I.D., Marcuzzi,G.P., Weissenborn,S.J., Kasper,H.U., Dries,V., Smyth,N., Fuchs,P. and Pfister,H. (2005) Development of skin tumors in mice transgenic for early genes of human papillomavirus type 8. *Cancer Res.*, **65**, 1394–1400.
34. Kim,S.H., Kim,K.S., Lee,E.J. *et al.* (2004) Human keratin 14 driven HPV 16 E6/E7 transgenic mice exhibit hyperkeratinosis. *Life Sci.*, **75**, 3035–3042.
35. Forslund,O., Lindelof,B., Hradil,E., Nordin,P., Stenquist,B., Kirnbauer,R., Slupetzky,K. and Dillner,J. (2004) High prevalence of cutaneous human papillomavirus DNA on the top of skin tumors but not in 'Stripped' biopsies from the same tumors. *J. Invest. Dermatol.*, **123**, 388–394.
36. Serrano,M., Hannon,G.J. and Beach,D. (1993) A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*, **366**, 704–707.
37. Sherr,C.J. (2004) Principles of tumor suppression. *Cell*, **116**, 235–246.
38. Kubo,Y., Urano,Y., Matsumoto,K., Ahsan,K. and Arase,S. (1997) Mutations of the INK4a locus in squamous cell carcinomas of human skin. *Biochem. Biophys. Res. Commun.*, **232**, 38–41.
39. Soufir,N., Daya-Grosjean,L., de La Salmoniere,P., Moles,J.P., Dubertret,L., Sarasin,A. and Basset-Seguin,N. (2000) Association between INK4a-ARF and p53 mutations in skin carcinomas of xeroderma pigmentosum patients. *J. Natl Cancer Inst.*, **92**, 1841–1847.
40. Soufir,N., Ribojad,M., Magnaldo,T., Thibaudeau,O., Delestaing,G., Daya-Grosjean,L., Rivet,J., Sarasin,A. and Basset-Seguin,N. (2002) Germline and somatic mutations of the INK4a-ARF gene in a xeroderma pigmentosum group C patient. *J. Invest. Dermatol.*, **119**, 1355–1360.
41. Quinn,A.G., Sikkink,S. and Rees,J.L. (1994) Delineation of two distinct deleted regions on chromosome 9 in human non-melanoma skin cancers. *Genes Chromosomes Cancer*, **11**, 222–225.
42. Klaes,R., Friedrich,T., Spitkovsky,D., Ridder,R., Rudy,W., Petry,U., Dallenbach-Hellweg,G., Schmidt,D. and von Knebel,D.M. (2001) Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *Int. J. Cancer*, **92**, 276–284.
43. Hodges,A. and Smoller,B.R. (2002) Immunohistochemical comparison of p16 expression in actinic keratoses and squamous cell carcinomas of the skin. *Mod. Pathol.*, **15**, 1121–1125.
44. Salama,M.E., Mahmood,M.N., Qureshi,H.S., Ma,C., Zarbo,R.J. and Ormsby,A.H. (2003) p16INK4a expression in actinic keratosis and Bowen's disease. *Br. J. Dermatol.*, **149**, 1006–1012.
45. Chan,T.A., Hermeking,H., Lengauer,C., Kinzler,K.W. and Vogelstein,B. (1999) 14-3-3Sigma is required to prevent mitotic catastrophe after DNA damage. *Nature*, **401**, 616–620.
46. Lodygin,D., Yazdi,A.S., Sander,C.A., Herzinger,T. and Hermeking,H. (2003) Analysis of 14-3-3sigma expression in hyperproliferative skin diseases reveals selective loss associated with CpG-methylation in basal cell carcinoma. *Oncogene*, **22**, 5519–5524.
47. van der Schroeff,J.G., Evers,L.M., Boot,A.J. and Bos,J.L. (1990) Ras oncogene mutations in basal cell carcinomas and squamous cell carcinomas of human skin. *J. Invest. Dermatol.*, **94**, 423–425.
48. Lieu,F.M., Yamanishi,K., Konishi,K., Kishimoto,S. and Yasuno,H. (1991) Low incidence of Ha-ras oncogene mutations in human epidermal tumors. *Cancer Lett.*, **59**, 231–235.
49. Pierceall,W.E., Goldberg,L.H., Tainsky,M.A., Mukhopadhyay,T. and Ananthaswamy,H.N. (1991) Ras gene mutation and amplification in human nonmelanoma skin cancers. *Mol. Carcinog.*, **4**, 196–202.
50. Campbell,C., Quinn,A.G. and Rees,J.L. (1993) Codon 12 Harvey-ras mutations are rare events in non-melanoma human skin cancer. *Br. J. Dermatol.*, **128**, 111–114.
51. Wilke,W.W., Robinson,R.A. and Kennard,C.D. (1993) H-ras-1 gene mutations in basal cell carcinoma: automated direct sequencing of clinical specimens. *Mod. Pathol.*, **6**, 15–19.
52. Spencer,J.M., Kahn,S.M., Jiang,W., DeLeo,V.A. and Weinstein,I.B. (1995) Activated ras genes occur in human actinic keratoses, premalignant precursors to squamous cell carcinomas. *Arch. Dermatol.*, **131**, 796–800.
53. Daya-Grosjean,L., Robert,C., Drougard,C., Suarez,H. and Sarasin,A. (1993) High mutation frequency in ras genes of skin tumors isolated from DNA repair deficient xeroderma pigmentosum patients. *Cancer Res.*, **53**, 1625–1629.
54. Dlugosz,A., Merlino,G. and Yuspa,S.H. (2002) Progress in cutaneous cancer research. *J. Investig. Dermatol. Symp. Proc.*, **7**, 17–26.
55. van Kranen,H.J. and de Gruijl,F.R. (1999) Mutations in cancer genes of UV-induced skin tumors of hairless mice. *J. Epidemiol.*, **9**, S58–S65.
56. de Gruijl,F.R. and Forbes,P.D. (1995) UV-induced skin cancer in a hairless mouse model. *Bioessays*, **17**, 651–660.
57. van Kranen,H.J., de Gruijl,F.R., de Vries,A., Sontag,Y., Wester,P.W., Senden,H.C., Rozemuller,E. and van Kreijl,C.F. (1995) Frequent p53 alterations but low incidence of ras mutations in UV-B-induced skin tumors of hairless mice. *Carcinogenesis*, **16**, 1141–1147.
58. Nishigori,C., Hattori,Y. and Toyokuni,S. (2004) Role of reactive oxygen species in skin carcinogenesis. *Antioxid. Redox. Signal.*, **6**, 561–570.
59. Gorlin,R.J. (1987) Nevoid basal-cell carcinoma syndrome. *Medicine (Baltimore)*, **66**, 98–113.
60. Shanley,S., Ratcliffe,J., Hockey,A., Haan,E., Oley,C., Ravine,D., Martin,N., Wicking,C. and Chenevix-Trench,G. (1994) Nevoid basal cell carcinoma syndrome: review of 118 affected individuals. *Am. J. Med. Genet.*, **50**, 282–290.
61. Kimonis,V.E., Goldstein,A.M., Pastakia,B., Yang,M.L., Kase,R., DiGiovanna,J.J., Bale,A.E. and Bale,S.J. (1997) Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. *Am. J. Med. Genet.*, **69**, 299–308.
62. Hahn,H., Wicking,C., Zaphiropoulous,P.G. *et al.* (1996) Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. *Cell*, **85**, 841–851.
63. Johnson,R.L., Rothman,A.L., Xie,J. *et al.* (1996) Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science*, **272**, 1668–1671.
64. Oro,A.E., Higgins,K.M., Hu,Z., Bonifas,J.M., Epstein,E.H.Jr and Scott,M.P. (1997) Basal cell carcinomas in mice overexpressing sonic hedgehog. *Science*, **276**, 817–821.
65. Aszterbaum,M., Epstein,J., Oro,A., Douglas,V., LeBoit,P.E., Scott,M.P. and Epstein,E.H.Jr (1999) Ultraviolet and ionizing radiation enhance the growth of BCCs and trichoblastomas in patched heterozygous knockout mice. *Nat. Med.*, **5**, 1285–1291.
66. Nilsson,M., Unden,A.B., Krause,D., Malmqwist,U., Raza,K., Zaphiropoulos,P.G. and Toftgard,R. (2000) Induction of basal cell

carcinomas and trichoepitheliomas in mice overexpressing GLI-1. *Proc. Natl Acad. Sci. USA*, **97**, 3438–3443.
67. Grachtchouk,M., Mo,R., Yu,S., Zhang,X., Sasaki,H., Hui,C.C. and Dlugosz,A.A. (2000) Basal cell carcinomas in mice overexpressing Gli2 in skin. *Nat. Genet.*, **24**, 216–217.
68. Fan,H. and Khavari,P.A. (1999) Sonic hedgehog opposes epithelial cell cycle arrest. *J. Cell Biol.*, **147**, 71–76.
69. Gailani,M.R., Stahle-Backdahl,M., Leffell,D.J. *et al*. (1996) The role of the human homologue of Drosophila patched in sporadic basal cell carcinomas. *Nat. Genet.*, **14**, 78–81.
70. Xie,J., Murone,M., Luoh,S.M. *et al*. (1998) Activating Smoothened mutations in sporadic basal-cell carcinoma. *Nature*, **391**, 90–92.
71. Jih,D.M., Lyle,S., Elenitsas,R., Elder,D.E. and Cotsarelis,G. (1999) Cytokeratin 15 expression in trichoepitheliomas and a subset of basal cell carcinomas suggests they originate from hair follicle stem cells. *J. Cutan. Pathol.*, **26**, 113–118.
72. Louro,I.D., Bailey,E.C., Li,X. *et al*. (2002) Comparative gene expression profile analysis of GLI and c-MYC in an epithelial model of malignant transformation. *Cancer Res.*, **62**, 5867–5873.
73. Ahmadian,A., Ren,Z.P., Williams,C., Ponten,F., Odeberg,J., Ponten,J., Uhlen,M. and Lundeberg,J. (1998) Genetic instability in the 9q22.3 region is a late event in the development of squamous cell carcinoma. *Oncogene*, **17**, 1837–1843.
74. Oro,A.E. and Higgins,K. (2003) Hair cycle regulation of Hedgehog signal reception. *Dev. Biol.*, **255**, 238–248.
75. Tsao,H. (2001) Genetics of nonmelanoma skin cancer. *Arch. Dermatol.*, **137**, 1486–1492.
76. Kallioniemi,A., Kallioniemi,O.P., Sudar,D., Rutovitz,D., Gray,J.W., Waldman,F. and Pinkel,D. (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science*, **258**, 818–821.
77. Jin,Y., Martins,C., Jin,C., Salemark,L., Jonsson,N., Persson,B., Roque,L., Fonseca,I. and Wennerberg,J. (1999) Nonrandom karyotypic features in squamous cell carcinomas of the skin. *Genes Chromosomes Cancer*, **26**, 295–303.
78. Popp,S., Waltering,S., Holtgreve-Grez,H., Jauch,A., Proby,C., Leigh,I.M. and Boukamp,P. (2000) Genetic characterization of a human skin carcinoma progression model: from primary tumor to metastasis. *J. Invest. Dermatol.*, **115**, 1095–1103.
79. Speicher,M.R., Gwyn,B.S. and Ward,D.C. (1996) Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat. Genet.*, **12**, 368–375.
80. Jin,Y., Martins,C., Salemark,L., Persson,B., Jin,C., Miranda,J., Fonseca,I. and Jonsson,N. (2001) Nonrandom karyotypic features in basal cell carcinomas of the skin. *Cancer Genet. Cytogenet.*, **131**, 109–119.
81. Larramendy,M.L., Koljonen,V., Bohling,T., Tukiainen,E. and Knuutila,S. (2004) Recurrent DNA copy number changes revealed by comparative genomic hybridization in primary Merkel cell carcinomas. *Mod. Pathol.*, **17**, 561–567.
82. Hitchcock,C.L., Bland,K.I., Laney,R.G.III, Franzini,D., Harris,B. and Copeland,E.M.III (1988) Neuroendocrine (Merkel cell) carcinoma of the skin. Its natural history, diagnosis, and treatment. *Ann. Surg.*, **207**, 201–207.
83. Proby,C.M., Purdie,K.J., Sexton,C.J., Purkis,P., Navsaria,H.A., Stables,J.N. and Leigh,I.M. (2000) Spontaneous keratinocyte cell lines representing early and advanced stages of malignant transformation of the epidermis. *Exp. Dermatol.*, **9**, 104–117.
84. Boukamp,P., Petrussevska,R.T., Breitkreutz,D., Hornung,J., Markham,A. and Fusenig,N.E. (1988) Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J. Cell Biol.*, **106**, 761–771.
85. Lehman,T.A., Modali,R., Boukamp,P., Stanek,J., Bennett,W.P., Welsh,J.A., Metcalf,R.A., Stampfer,M.R., Fusenig,N. and Rogan,E.M. (1993) p53 mutations in human immortalized epithelial cell lines. *Carcinogenesis*, **14**, 833–839.
86. Boukamp,P., Popp,S., Altmeyer,S., Hulsen,A., Fasching,C., Cremer,T. and Fusenig,N.E. (1997) Sustained nontumorigenic phenotype correlates with a largely stable chromosome content during long-term culture of the human keratinocyte line HaCaT. *Genes Chromosomes Cancer*, **19**, 201–214.
87. Boukamp,P., Stanbridge,E.J., Foo,D.Y., Cerutti,P.A. and Fusenig,N.E. (1990) c-Ha-ras oncogene expression in immortalized human keratinocytes (HaCaT) alters growth potential *in vivo* but lacks correlation with malignancy. *Cancer Res.*, **50**, 2840–2847.
88. Boukamp,P., Peter,W., Pascheberg,U., Altmeier,S., Fasching,C., Stanbridge,E.J. and Fusenig,N.E. (1995) Step-wise progression in human skin carcinogenesis *in vitro* involves mutational inactivation of p53, rasH oncogene activation and additional chromosome loss. *Oncogene*, **11**, 961–969.
89. Mueller,M.M., Peter,W., Mappes,M., Huelsen,A., Steinbauer,H., Boukamp,P., Vaccariello,M., Garlick,J. and Fusenig,N.E. (2001) Tumor progression of skin carcinoma cells *in vivo* promoted by clonal selection, mutagenesis, and autocrine growth regulation by granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. *Am. J. Pathol.*, **159**, 1567–1579.
90. Boukamp,P., Popp,S., Bleuel,K., Tomakidi,E., Burkle,A. and Fusenig,N.E. (1999) Tumorigenic conversion of immortal human skin keratinocytes (HaCaT) by elevated temperature. *Oncogene*, **18**, 5638–5645.
91. Skobe,M. and Fusenig,N.E. (1998) Tumorigenic conversion of immortal human keratinocytes through stromal cell activation. *Proc. Natl Acad. Sci. USA*, **95**, 1050–1055.
92. Mueller,M.M. and Fusenig,N.E. (1999) Constitutive expression of G-CSF and GM-CSF in human skin carcinoma cells with functional consequence for tumor progression. *Int. J. Cancer*, **83**, 780–789.
93. Mudgil,A.V., Segal,N., Andriani,F., Wang,Y., Fusenig,N.E. and Garlick,J.A. (2003) Ultraviolet B irradiation induces expansion of intraepithelial tumor cells in a tissue model of early cancer progression. *J. Invest. Dermatol.*, **121**, 191–197.
94. Billings,S.D., Southall,M.D., Li,T., Cook,P.W., Baldridge,L., Moores,W.B., Spandau,D.F., Foley,J.G. and Travers,J.B. (2003) Amphiregulin overexpression results in rapidly growing keratinocytic tumors: an *in vivo* xenograft model of keratoacanthoma. *Am. J. Pathol.*, **163**, 2451–2458.
95. Duesberg,P.H. (2003) Are cancers dependent on oncogenes or on aneuploidy? *Cancer Genet. Cytogenet.*, **143**, 89–91.
96. Lane,D.P. (1992) Cancer. p53, guardian of the genome. *Nature*, **358**, 15–16.
97. Vogelstein,B., Lane,D. and Levine,A.J. (2000) Surfing the p53 network. *Nature*, **408**, 307–310.
98. Georgiades,I.B., Curtis,L.J., Morris,R.M., Bird,C.C. and Wyllie,A.H. (1999) Heterogeneity studies identify a subset of sporadic colorectal cancers without evidence for chromosomal or microsatellite instability. *Oncogene*, **18**, 7933–7940.
99. Eyfjord,J.E., Thorlacius,S., Steinarsdottir,M., Valgardsdottir,R., Ogmundsdottir,H.M. and Anamthawat-Jonsson,K. (1995) p53 abnormalities and genomic instability in primary human breast carcinomas. *Cancer Res.*, **55**, 646–651.
100. Primdahl,H., Wikman,F.P., von der,M.H., Zhou,X.G., Wolf,H. and Orntoft,T.F. (2002) Allelic imbalances in human bladder cancer: genome-wide detection with high-density single-nucleotide polymorphism arrays. *J. Natl Cancer Inst.*, **94**, 216–223.
101. Overholtzer,M., Rao,P.H., Favis,R., Lu,X.Y., Elowitz,M.B., Barany,F., Ladanyi,M., Gorlick,R. and Levine,A.J. (2003) The presence of p53 mutations in human osteosarcomas correlates with high levels of genomic instability. *Proc. Natl Acad. Sci. USA*, **100**, 11547–11552.
102. Carroll,P.E., Okuda,M., Horn,H.F., Biddinger,P., Stambrook,P.J., Gleich,L.L., Li,Y.Q., Tarapore,P. and Fukasawa,K. (1999) Centrosome hyperamplification in human cancer: chromosome instability induced by p53 mutation and/or Mdm2 overexpression. *Oncogene*, **18**, 1935–1944.
103. Hinchcliffe,E.H. and Sluder,G. (2002) Two for two: Cdk2 and its role in centrosome doubling. *Oncogene*, **21**, 6154–6160.
104. Kramer,A., Neben,K. and Ho,A.D. (2002) Centrosome replication, genomic instability and cancer. *Leukemia*, **16**, 767–775.
105. White,A.E., Livanos,E.M. and Tlsty,T.D. (1994) Differential disruption of genomic integrity and cell cycle regulation in normal human fibroblasts by the HPV oncoproteins. *Genes Dev.*, **8**, 666–677.
106. Hashida,T. and Yasumoto,S. (1991) Induction of chromosome abnormalities in mouse and human epidermal keratinocytes by the human papillomavirus type 16 E7 oncogene. *J. Gen. Virol.*, **72** (Pt 7), 1569–1577.
107. Duensing,S., Lee,L.Y., Duensing,A., Basile,J., Piboonniyom,S., Gonzalez,S., Crum,C.P. and Munger,K. (2000) The human papillomavirus type 16 E6 and E7 oncoproteins cooperate to induce mitotic defects and genomic instability by uncoupling centrosome duplication from the cell division cycle. *Proc. Natl Acad. Sci. USA*, **97**, 10002–10007.
108. Duensing,S., Duensing,A., Crum,C.P. and Munger,K. (2001) Human papillomavirus type 16 E7 oncoprotein-induced abnormal centrosome synthesis is an early event in the evolving malignant phenotype. *Cancer Res.*, **61**, 2356–2360.
109. McClintock,B. (1941) The stability of broken ends of chromosomes in Zea mays. *Genetics*, **26**, 234–282.

110. Blackburn,E.H. (2001) Switching and signaling at the telomere. *Cell*, **106**, 661–673.
111. Griffith,J.D., Comeau,L., Rosenfield,S., Stansel,R.M., Bianchi,A., Moss,H. and de Lange,T. (1999) Mammalian telomeres end in a large duplex loop. *Cell*, **97**, 503–514.
112. Olovnikov,A.M. (1973) A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J. Theor. Biol.*, **41**, 181–190.
113. Wright,W.E. and Shay,J.W. (2002) Historical claims and current interpretations of replicative aging. *Nat. Biotechnol.*, **20**, 682–688.
114. Blasco,M.A., Lee,H.W., Rizen,M., Hanahan,D., DePinho,R. and Greider,C.W. (1997) Mouse models for the study of telomerase. *Ciba Found. Symp.*, **211**, 160–170.
115. Hemann,M.T., Strong,M.A., Hao,L.Y. and Greider,C.W. (2001) The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell*, **107**, 67–77.
116. van Steensel,B., Smogorzewska,A. and de Lange,T. (1998) TRF2 protects human telomeres from end-to-end fusions. *Cell*, **92**, 401–413.
117. Chang,S., Khoo,C. and DePinho,R.A. (2001) Modeling chromosomal instability and epithelial carcinogenesis in the telomerase-deficient mouse. *Semin. Cancer Biol.*, **11**, 227–239.
118. Murnane,J.P. and Sabatier,L. (2004) Chromosome rearrangements resulting from telomere dysfunction and their role in cancer. *Bioessays*, **26**, 1164–1174.
119. Hackett,J.A. and Greider,C.W. (2003) End resection initiates genomic instability in the absence of telomerase. *Mol. Cell. Biol.*, **23**, 8450–8461.
120. Goytisolo,F.A. and Blasco,M.A. (2002) Many ways to telomere dysfunction: *in vivo* studies using mouse models. *Oncogene*, **21**, 584–591.
121. Bailey,S.M. and Goodwin,E.H. (2004) DNA and telomeres: beginnings and endings. *Cytogenet. Genome Res.*, **104**, 109–115.
122. Espejel,S., Klatt,P., Menissier-de Murcia,J., Martin-Caballero,J., Flores,J.M., Taccioli,G., de Murcia,G. and Blasco,M.A. (2004) Impact of telomerase ablation on organismal viability, aging, and tumorigenesis in mice lacking the DNA repair proteins PARP-1, Ku86, or DNA-PKcs. *J. Cell Biol.*, **167**, 627–638.
123. Rudolph,K.L., Chang,S., Lee,H.W., Blasco,M., Gottlieb,G.J., Greider,C. and DePinho,R.A. (1999) Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell*, **96**, 701–712.
124. Gonzalez-Suarez,E., Samper,E., Flores,J.M. and Blasco,M.A. (2000) Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. *Nat. Genet.*, **26**, 114–117.
125. Argilla,D., Chin,K., Singh,M., Hodgson,J.G., Bosenberg,M., de Solorzano,C.O., Lockett,S., DePinho,R.A., Gray,J. and Hanahan,D. (2004) Absence of telomerase and shortened telomeres have minimal effects on skin and pancreatic carcinogenesis elicited by viral oncogenes. *Cancer Cell*, **6**, 373–385.
126. Artandi,S.E., Alson,S., Tietze,M.K. *et al.* (2002) Constitutive telomerase expression promotes mammary carcinomas in aging mice. *Proc. Natl Acad. Sci. USA*, **99**, 8191–8196.
127. Gonzalez-Suarez,E., Samper,E., Ramirez,A., Flores,J.M., Martin-Caballero,J., Jorcano,J.L. and Blasco,M.A. (2001) Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes. *EMBO J.*, **20**, 2619–2630.
128. Gonzalez-Suarez,E., Flores,J.M. and Blasco,M.A. (2002) Cooperation between p53 mutation and high telomerase transgenic expression in spontaneous cancer development. *Mol. Cell. Biol.*, **22**, 7291–7301.
129. Mai,S. and Mushinski,J.F. (2003) c-Myc-induced genomic instability. *J. Environ. Pathol. Toxicol. Oncol.*, **22**, 179–199.
130. Pelisson,I., Soler,C., Chardonnet,Y., Euvrard,S. and Schmitt,D. (1996) A possible role for human papillomaviruses and c-myc, c-Ha-ras, and p53 gene alterations in malignant cutaneous lesions from renal transplant recipients. *Cancer Detect. Prev.*, **20**, 20–30.
131. Mai,S., Fluri,M., Siwarski,D. and Huppi,K. (1996) Genomic instability in MycER-activated Rat1A-MycER cells. *Chromosome Res.*, **4**, 365–371.
132. Felsher,D.W. and Bishop,J.M. (1999) Transient excess of MYC activity can elicit genomic instability and tumorigenesis. *Proc. Natl Acad. Sci. USA*, **96**, 3940–3944.
133. Kuschak,T.I., Taylor,C., McMillan-Ward,E., Israels,S., Henderson,D.W., Mushinski,J.F., Wright,J.A. and Mai,S. (1999) The ribonucleotide reductase R2 gene is a non-transcribed target of c-Myc-induced genomic instability. *Gene*, **238**, 351–365.
134. McCormack,S.J., Weaver,Z., Deming,S., Natarajan,G., Torri,J., Johnson,M.D., Liyanage,M., Ried,T. and Dickson,R.B. (1998) Myc/p53 interactions in transgenic mouse mammary development, tumorigenesis and chromosomal instability. *Oncogene*, **16**, 2755–2766.
135. Chuang,T.C., Moshir,S., Garini,Y. *et al.* (2004) The three-dimensional organization of telomeres in the nucleus of mammalian cells. *BMC Biol.*, **2**, 12.
136. Ermler,S., Krunic,D., Knoch,T.A., Moshir,S., Mai,S., Greulich-Bode,K.M. and Boukamp,P. (2004) Cell cycle-dependent 3D distribution of telomeres and TRF2 in HaCaT and HaCaT-myc cells. *Eur. J. Cell Biol.*, **83**, 681–690.
137. Popp,S., Waltering,S., Holtgreve-Grez,H., Jauch,A., Proby,C., Leigh,I.M. and Boukamp,P. (2000) Genetic characterization of a human skin carcinoma progression model: from primary tumor to metastasis. *J. Invest Dermatol.*, **115**, 1095–1103.
138. Murphy,G., Young,A.R., Wulf,H.C., Kulms,D. and Schwarz,T. (2001) The molecular determinants of sunburn cell formation. *Exp. Dermatol.*, **10**, 155–160.
139. Clark,W.H.Jr, Elder,D.E., Guerry,D., Braitman,L.E., Trock,B.J., Schultz,D., Synnestvedt,M. and Halpern,A.C. (1989) Model predicting survival in stage I melanoma based on tumor progression. *J. Natl Cancer Inst.*, **81**, 1893–1904.
140. Ciullo,M., Debily,M.A., Rozier,L. *et al.* (2002) Initiation of the breakage-fusion-bridge mechanism through common fragile site activation in human breast cancer cells: the model of PIP gene duplication from a break at FRA7I. *Hum. Mol. Genet.*, **11**, 2887–2894.
141. Hellman,A., Zlotorynski,E., Scherer,S.W., Cheung,J., Vincent,J.B., Smith,D.I., Trakhtenbrot,L. and Kerem,B. (2002) A role for common fragile site induction in amplification of human oncogenes. *Cancer Cell*, **1**, 89–97.
142. Huebner,K. and Croce,C.M. (2003) Cancer and the FRA3B/FHIT fragile locus: it's a HIT. *Br. J. Cancer*, **88**, 1501–1506.
143. Sikkink,S.K., Rehman,I. and Rees,J.L. (1997) Deletion mapping of chromosome 3p and 13q and preliminary analysis of the FHIT gene in human nonmelanoma skin cancer. *J. Invest Dermatol.*, **109**, 801–805.
144. Rief,N. and Lobrich,M. (2002) Efficient rejoining of radiation-induced DNA double-strand breaks in centromeric DNA of human cells. *J. Biol. Chem.*, **277**, 20572–20582.
145. Slijepcevic,P. and Natarajan,A.T. (1994) Distribution of X-ray-induced G2 chromatid damage among Chinese hamster chromosomes: influence of chromatin conformation. *Mutat. Res.*, **323**, 113–119.
146. Muhlmann-Diaz,M.C. and Bedford,J.S. (1994) Breakage of human chromosomes 4, 19 and Y in G0 cells immediately after exposure to gamma-rays. *Int. J. Radiat. Biol.*, **65**, 165–173.
147. Surralles,J., Darroudi,F. and Natarajan,A.T. (1997) Low level of DNA repair in human chromosome 1 heterochromatin. *Genes Chromosomes Cancer*, **20**, 173–184.
148. Barbouti,A., Stankiewicz,P., Nusbaum,C. *et al.* (2004) The breakpoint region of the most common isochromosome, i(17q), in human neoplasia is characterized by a complex genomic architecture with large, palindromic, low-copy repeats. *Am. J. Hum. Genet.*, **74**, 1–10.
149. Dej,K.J. and Orr-Weaver,T.L. (2000) Separation anxiety at the centromere. *Trends Cell Biol.*, **10**, 392–399.
150. Taipale,J., Chen,J.K., Cooper,M.K., Wang,B., Mann,R.K., Milenkovic,L., Scott,M.P. and Beachy,P.A. (2000) Effects of oncogenic mutations in Smoothened and Patched can be reversed by cyclopamine. *Nature*, **406**, 1005–1009.
151. Nishigori,C., Hattori,Y. and Toyokuni,S. (2004) Role of reactive oxygen species in skin carcinogenesis. *Antioxid. Redox. Signal.*, **6**, 561–570.
152. Phillipson,R.P., Tobi,S.E., Morris,J.A. and McMillan,T.J. (2002) UV-A induces persistent genomic instability in human keratinocytes through an oxidative stress mechanism. *Free Radic. Biol. Med.*, **32**, 474–480.

Received May 4, 2005; revised and accepted May 10, 2005