

REVIEW

Molecular carcinogenesis of squamous cell carcinomas of the skin

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Abstract : Squamous cell carcinomas (SCCs) of the skin were suggested to develop through a multistep process that involves activation of proto-oncogenes and/or inactivation of tumor suppressor genes in the human skin keratinocytes. Exposure to ultra-violet (UV), especially UV-B, radiation is the most common cause for these genetic abnormalities in cells. We review causation of SCCs and genetic abnormalities in human SCCs with the current work. To elucidate the multistep process, we developed a method for examining the combinatorial function *in vivo* of plural genes in human keratinocytes. Using high efficiency retroviral transductions, we could express plural genes serially in normal human primary keratinocytes and use these cells to regenerate human skin on SCID mice. A combinatorial transduction of H-RasV12 and cyclin dependent kinase 4 (CDK4) produced human epidermal neoplasia resembling SCC. These findings were consistent with our previous results of mutation analysis in SCCs, one of which had both mutations of *H-Ras* gene and the *INK4a* locus. Therefore, it is suggested that a combination of these genetic abnormalities might be crucial to the carcinogenesis at least in a subset of SCCs. *J. Med. Invest.* **49** : 111-117, 2002

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INTRODUCTION

Squamous cell carcinomas (SCCs) of the skin are one of the most common skin cancers associated with a substantial risk of metastasis (1). It is widely accepted that normal keratinocytes in the epidermis can convert to SCCs through a multistep process that involves activation of proto-oncogenes and/or inactivation of tumor suppressor genes (2, 3). In this study, we review the molecular carcinogenesis of SCCs.

CAUSATION

Exposure to ultra-violet (UV) radiation is the most common cause of skin cancers (4, 5). In particular, UV-B radiation is mainly involved in the mutagenesis in the skin by two major photoproducts of a cyclobutane pyrimidine dimer and (6-4) photoproducts (6, 7). After UV-B radiation exposure, DNA damage in the keratinocytes would usually be repaired properly by means of DNA base excision repair. However, the abnormalities of genes would remain in the cells, if the cells fail to undergo DNA damage repair they could escape from apoptosis. Recently, tanning devices have been reported to be associated with odds ratios of 2.5 for SCCs in Caucasians (8).

The hypercarcinogenic state has been defined as the state that cells are susceptible to the occurrence and accumulation of gene mutations (9). Hypercarcinogenic

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states for SCCs consist of inherited lesions, e.g., xeroderma pigmentosum that is defective in DNA base excision repair, and the acquired lesions, e.g., chronic ulcers, burn and posttraumatic scars, and Discoid lupus erythematosus. Among them, each of the candidate genes for all types of xeroderma pigmentosum has previously been identified, and the mechanism of DNA base excision repair has been elucidated (10). Although how gene mutations accumulate in the acquired lesions is poorly understood, the continually accelerated turnover of the cell cycle in chronic ulcers was suggested to be one of the most important factors for the initiation, promotion, and progression of carcinogenesis as the hypercarcinogenic state (9).

SCCs occur with high frequency in renal allograft recipients after prolonged immunosuppression (11). Human papillomavirus (HPV) infections are likely to be an important factor because they are often detected in these conditions (12, 13). In oncogenic types of HPV 16 and 18, which are frequently detected in uterocervical cancers, E6 protein inactivates the p53 tumor suppressor gene product and up-regulates hTERT, the reverse transcriptase of telomerase, and E7 protein inactivates Rb (Retinoblastoma) tumor suppressor gene product (14). It may be uncertain that HPV should mainly contribute to the development of SCCs because HPV 16 or 18 are not often detected in those on the immunosuppressive patients. However, E6 proteins of other types of HPV detected frequently in the skin also target and abrogate the function of Bak, an apoptosis-related protein that is induced by UV (15). Thus, HPV may contribute to the development of SCCs against apoptotic signals in keratinocytes.

Some gene polymorphisms related to the susceptibility of SCCs were reported in the general population of Caucasians recently. Greater than twenty polymorphisms were reported in the melanocortin-1 receptor (MC1-R), which has been associated with physiologic variation in hair and skin color. In particular, the variants Arg151Cys and Arg160Trp were strongly associated with fair skin and red hair, and carriers of the two variant alleles were at increased risk for developing SCCs (16). The DNA base excision repair gene XRCC1 Arg399Gln polymorphism was also reported to be associated with the occurrence of SCCs (17). These associations between gene polymorphisms and the occurrence of SCCs remain uncertain in the general Japanese population.

GENETIC ABNORMALITIES

1) Chromosomal abnormalities

The majority of a number of DNA ploidy studies reveal that aneuploidy is shown in some SCCs (18). Consistent with these findings, clonal chromosomal abnormalities have been reported in a subset of SCCs, although it is technically difficult to perform karyotypic analysis in solid tumors (19). Chromosomal instabilities may be critical on the carcinogenesis in a subset of SCCs, although chromosomal instabilities as a result of transformation or technical artifacts could not be completely excluded.

2) Proto-oncogene *Ras*

Ras genes consist of three different genes, *H-Ras*, *K-Ras*, and *N-Ras*, and activating *Ras* mutations are one of the most common genetic abnormalities in various human cancers (20). The *Ras* proteins are small G-proteins that transduce intracellular signal, and are constitutively activated by point mutations of codons 12, 13, and 61 of *Ras* genes (20, 21). Through the activated *Ras* pathway, many tumor-promoting effects, e.g., accelerating cell growth and inhibiting apoptosis, are induced (21). *Ras* mutations have been well characterized in the mouse skin two-stage carcinogenesis model, because the mutations are frequently detected after the initiation with the genotoxic carcinogen dimethylbenzanthracene (DMBA) (22, 23). However, the rates of *Ras* mutations in human SCCs vary between 0% and 46% (24, 25), and we also detected *Ras* mutations in only one of 21 SCC cases (Table 1) (26). Activating *Ras* mutations should be important on the carcinogenesis in a subset of SCCs, although the role of *Ras* activation in SCCs development remains unclear.

3) Tumor suppressor gene *p53*

Mutations of the *p53* gene have been found in approximately half the SCC cases in addition to various other human cancers (27-29). UV light-induced photoproducts at dipyrimidine sites should contribute to these mutations, because mutations of C to T or CC to TT predominate in SCCs that originate in the sunlight-exposed skin region (27-29). Known as "guardian of the genome" (30), *p53* is involved in a number of important cellular control pathways, including G1 growth arrest and apoptosis, especially in response to DNA damage. *p53* induces p21 and p53R2 for repairing DNA damage in the cells during G1 growth arrest (31, 32), and induces Bax and

Table 1. Summary of gene mutations in 21 SCCs

| Age (Gender) | Predisposing conditions | TNM | <i>H-Ras</i> | <i>p53</i> | <i>p16^{INK4a}</i> | <i>p14^{ARF}</i> |
|--------------|-------------------------|-----|--------------|-----------------------|----------------------------|--------------------------|
| 76 (M) | | I | - | - | CC T | CC T |
| 93 (F) | | II | - | Y107X, C G | - | - |
| 79 (M) | Sun exposure | II | - | G244S, C T | - | - |
| 73 (M) | | II | - | Q317X, C T | - | - |
| 93 (F) | | II | - | R249T, C G | - | - |
| 63 (F) | | II | G13R, C G | - | 21bp deletion | 21bp deletion |
| 48 (F) | Scar | III | - | Y234X, C A | R80X, C T | P94L, C T |
| 88 (F) | Scar | II | - | E326X, C A | - | - |
| 68 (M) | Radiation dermatitis | II | - | H178Q, H179Y CC AT | - | - |

p53AIP1 for rendering cells apoptotic if huge DNA damage remains in the cells (33, 34). The cells where the p53 functions are lost would render them resistant to cell growth arrest and apoptosis, and they would be susceptible to the occurrence and accumulation of gene mutations in addition to accelerated cell growth. Since mutations of the *p53* gene have been found in lesions of solar keratosis and apparently histological normal skin (35-38), the mutations might occur at an early stage in the development of SCCs.

4) The *INK4a* locus

The *INK4a* locus encodes two different tumor suppressor gene products, p16^{INK4a} and p14^{ARF} (39, 40). Each has its own promoter and exon 1, and shares the same exon 2 with different reading frames from each other (39, 40). p16^{INK4a} is involved in the function of cell growth suppression of Rb by binding cyclin dependent kinase 4/6 (CDK4/6) and inhibiting their enzyme activities (41), and p14^{ARF} is involved in the function of cell growth arrest and apoptosis of p53 by binding MDM2 and stabilizing p53 (40). Mutations of the *INK4a* locus have been reported in up to 20% of SCCs (42, 43). They have so far been detected in exon 2, which is common to both *p16^{INK4a}* and *p14^{ARF}* (42, 43). Although expression of the catalytic component of telomerase, hTERT, alone is sufficient for immortalizing human fibroblasts, both Rb/ p16^{INK4a} inactivation and hTERT are required to immortalize human keratinocytes (44). These findings suggest that Rb/ p16^{INK4a} inactivation might have some relevance to the carcinogenesis in some of SCCs

5) Allelic loss

Tumor suppressor genes have been revealed by

the study of hereditary human cancers (45). Although these genes render carriers heterozygous and so appear in pedigrees, as dominantly inherited disorders, they were suggested to be recessive in carcinogenesis (45). "Knudson's two-hit hypothesis" that inactivation of both maternal and paternal alleles should be essential in carcinogenesis is widely accepted. In many instances, one allele is mutated and another allele is lost although there are exceptions. Allelic loss can be detected in tumor tissues by means of PCR assays based on microsatellite sequences, which are widely dispersed throughout the genome and usually highly informative. Although allelic loss in SCCs has been found on many chromosomes, the rates of allelic loss are relative high, in approximately 20% to 40% of SCCs, on 3p, 9p, 9q, 13q, 17p, and 17 q (46-48). The *INK4a* locus, *Rb* gene, and *p53* gene are located on 9p, 13q, and 17p, respectively. Recently, the gene responsible for multiple self-healing squamous epithelioma syndrome (Ferguson-Smith) was mapped on 9q22, which is expected to be identified (49).

THE MULTI-STEP PROCESS IN CARCINOGENESIS

Although many studies regarding chromosomal and genetic abnormalities in SCCs have been reported, the multi-step process in carcinogenesis of SCCs is still unclear. We have been trying to examine the combinatorial function of activation of proto-oncogenes and inactivation of tumor suppressor genes in normal human keratinocytes (Figure 1), especially activation of *H-Ras* and inactivation of the *INK4a* locus, because we found one SCC with both mutations of these two genes (26). Since neither dominant nega-

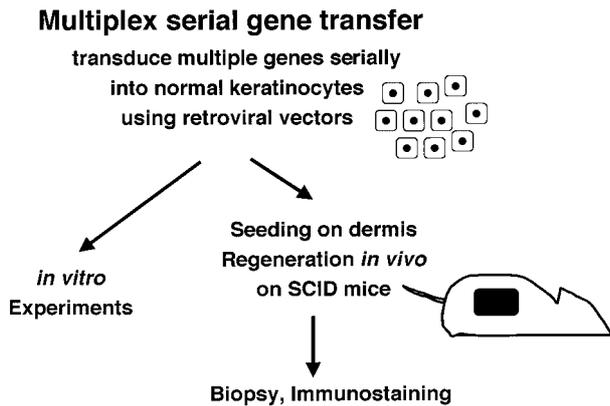


Fig. 1. Experimental procedure.

tive mutant p16^{INK4a} nor p14^{ARF} in the *INK4a* locus were found, activation of CDK4 and inactivation of p53 were substituted for inactivation of p16^{INK4a} and p14^{ARF}, respectively. Using high efficiency retroviral transductions in normal human primary keratinocytes (50), we expressed H-RasV12 (an activated mutant H-Ras), CDK4, p53W248 (a dominant-negative mutant p53), and hTERT either singly or in combination and used these cells to regenerate human skin on SCID mice.

A combination of H-RasV12 and CDK4 produced human skin tumors with histologic features of SCC at 7 weeks after grafting, although a combination of H-RasV12 and p53W248 showed no specific effects compared with normal controls (Figure 2) (51). The tumors derived from the cells where both H-RasV12 and CDK4 were transduced (Ras-CDK4 tumors), similar to human SCCs, expressed increased levels of Cyclin D1 and VEGF. Cyclin D1 was necessary but not sufficient for Ras-CDK4 tumors, because a combination of Cyclin D 1 and CDK 4 failed to induce tu-

mors while an anti-sense Cyclin D 1 retrovector that suppressed D1 tissue protein expression abolished Ras-CDK4 tumors (51). In addition, CDK4 synergy with H-RasV12 is dependent on intrinsic CDK4 kinase function because the kinase-dead N158 CDK4 point mutant failed to induce tumors when co-expressed with H-RasV12 (51). These findings identify Ras and CDK4 as capable of converting normal human epidermal tissue into invasive neoplasia and suggest that functional CDK4 and Cyclin D1 is necessary for this process. Thus, it is suggested that a combination of Ras activation and Rb/ p16^{INK4a} inactivation might be crucial to the carcinogenesis at least in a subset of SCCs.

CONCLUDING REMARKS

Squamous cell carcinomas (SCCs) of the skin are one of the most common skin disorders, and sometimes recur or metastasize after surgical excision. Advanced SCCs are often resistant to radiation treatment and chemotherapy. We hope that molecular carcinogenesis of SCCs of the skin would be elucidated in the near future to establish some markers for the prognosis of SCCs and a novel effective therapy for advanced SCCs.

REFERENCES

1. Alam M, Ratner D : Cutaneous squamous cell carcinoma. *N Engl J Med* 344 : 975-983, 2001
2. Rees JL : Part III Squamous cell carcinoma. *Molecular biology*. In : Miller SJ, Maloney ME,

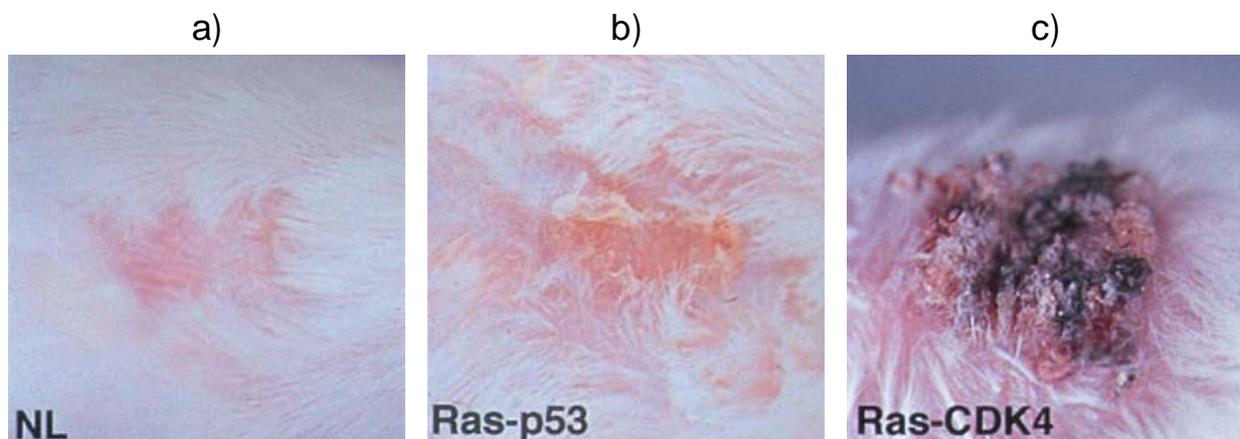


Fig. 2. Clinical features at 7 weeks after grafting. a) green fluorescent protein, b) both of H-RasV12 and p53W248, and c) both of H-RasV12 and CDK4 were transduced into keratinocytes for regenerating human skin on SCID mice, respectively. Note a) the smooth appearance and b) the slightly scaly surface of the skin in contrast to c) the large tumor.

- eds. Cutaneous Oncology. Blackwell Science Inc, Malden, 1998, pp. 353-360
3. Fusenig NE, Boukamp P : Multiple stages and genetic alterations in immortalization, malignant transformation, and tumor progression of human skin keratinocytes. *Mol Carcinog* 23 : 144-58, 1998
 4. Grossman D, Leffell DJ : The molecular basis of nonmelanoma skin cancer : new understanding. *Arch Dermatol* 133 : 1263-70, 1997
 5. de Grujil FR : Skin cancer and solar UV radiation. *Eur J Cancer* 35 : 2003-9, 1999
 6. Protic-Sabljić M, Tuteja N, Munson PJ, Hauser J, Kraemer KH, Dixon K : UV light-induced cyclobutane pyrimidine dimers are mutagenic in mammalian cells. *Mol Cell Biol* 6 : 3349-56, 1986
 7. Brash DE, Ziegler A, Jonason AS, Simon JA, Kunala S, Leffell DJ : Sunlight and sunburn in human skin cancer : p53, apoptosis, and tumor promotion. *J Invest Dermatol Symp Proc* 1 : 136-142, 1996
 8. Karagas MR, Stannard VA, Mott LA, Slattery MJ, Spencer SK, Weinstock MA : Use of tanning devices and risk of basal cell and squamous cell skin cancers. *J Natl Cancer Inst* 94 : 224-226, 2002
 9. Kitagawa T : Definition of hypercarcinogenic lesion (state). *Mol Med* 32 : 1146-1148, 1995
 10. Cleaver JE : Common pathways for ultraviolet skin carcinogenesis in the repair and replication defective groups of xeroderma pigmentosum. *J Dermatol Sci* 23 : 1-11, 2000
 11. Euvrard S, Chardonnet Y, Pouteil-Noble C, Kanitakis J, Chignol MC, Thivolet J, Touraine JL : Association of skin malignancies with various and multiple carcinogenic and noncarcinogenic human papillomaviruses in renal transplant recipients. *Cancer* 72 : 2198-2206, 1993
 12. Bouwes Bavinck J N, Feltkamp M, Struijk L, ter Scheggett J : Human papillomavirus infection and skin cancer risk in organ transplant recipients. *J Invest Dermatol Symp Proc* 6 : 207-11, 2001
 13. Shamanin V, zur Hausen H, Lavergne D, Proby CM, Leigh IM, Neumann C, Hamm H, Goos M, Haustein UF, Jung EG, Plewig G, Wolff H, de Villiers E-M : Human papillomavirus infections in nonmelanoma skin cancers from renal transplant recipients and nonimmunosuppressed patients. *J Natl Cancer Inst* 88 : 802-811, 1996
 14. Galloway DA, McDougall JK : The disruption of cell cycle checkpoints by papillomavirus oncoproteins contributes to anogenital neoplasia. *Semin Cancer Biol* 7 : 309-315, 1996
 15. Jackson S, Harwood C, Thomas M, Banks L, Storey A : Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. *Genes Dev* 14 : 3065-3073, 2000
 16. Bastiaens MT, ter Huurne JA, Kielich C, Gruis NA, Westendorp RG, Vermeer BJ, Bavinck JN, The Leiden Skin Cancer Study Team : Melanocortin-1 receptor gene variants determine the risk of nonmelanoma skin cancer independently of fair skin and red hair. *Am J Hum Genet* 68 : 884-894, 2001
 17. Nelson HH, Kelsey KT, Mott LA, Karagas MR : The XRCC1 Arg399Gln polymorphism, sunburn, and non-melanoma skin cancer : Evidence of gene-environment interaction. *Cancer Res* 62 : 152-155, 2002
 18. Robinson JK, Rademaker AW, Goolsby C, Traczyk TN, Zoladz C : DNA ploidy in nonmelanoma skin cancer. *Cancer* 77 : 284-291, 1996
 19. Jin Y, Martins C, Jin C, Salemark L, Jonsson N, Persson B, Roque L, Fonseca I, Wennerberg J : Nonrandom karyotypic features in squamous cell carcinomas of the skin. *Genes Chromosomes Cancer* 26 : 295-303, 1999
 20. Bos JL : The ras gene family and human carcinogenesis. *Mutat Res* 195 : 255-71, 1988
 21. Shields JM, Pruitt K, McFall A, Shaub A, Der CJ : Understanding Ras : 'it ain't over 'til it's over'. *Trends Cell Biol* 10 : 147-54, 2000
 22. Rodriguez-Puebla ML, Robles AI, Conti CJ : ras activity and cyclin D1 expression : an essential mechanism of mouse skin tumor development. *Mol Carcinog* 24 : 1-6, 1999
 23. Ise K, Nakamura K, Nakao K, Shimizu S, Harada H, Ichise T, Miyoshi J, Gondo Y, Ishikawa T, Aiba A, Katsuki M : Targeted deletion of the H-ras gene decreases tumor formation in mouse skin carcinogenesis. *Oncogene* 19 : 2951-6, 2000
 24. van der Schroeff JG, Evers LM, Boot AJ, Bos JL : Ras oncogene mutations in basal cell carcinomas and squamous cell carcinomas of human skin. *J Invest Dermatol* 94 : 423-5, 1990
 25. Pierceall WE, Goldberg LH, Tainsky MA, Mukhopadhyay T, Ananthaswamy HN : Ras gene mutation and amplification in human nonmelanoma skin cancers. *Mol Carcinog* 4 : 196-202, 1991
 26. Kubo Y, Urano Y, Matsumoto K, Arase S : Mutations of cancer-related genes in squamous cell carcinomas of the human skin. *Skin Cancer*

- 16 : 353-356, 2001 (in Japanese)
27. Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, Halperin AJ, Ponten J : A role for sunlight in skin cancer : UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci USA* 88 : 10124-8, 1991
 28. Moles JP, Moyret C, Guillot B, Jeanteur P, Guilhou JJ, Theillet C, Basset-Seguain N : p53 gene mutations in human epithelial skin cancers. *Oncogene* 8 : 583-8, 1993
 29. Kubo Y, Urano Y, Yoshimoto K, Iwahana H, Fukuhara K, Arase S, Itakura M : p53 gene mutations in human skin cancers and precancerous lesions : Comparison with immunohistochemical analysis. *J Invest Dermatol* 102 : 440-444, 1994
 30. Lane DP : Cancer. p 53, guardian of the genome. *Nature* 358 : 15-16, 1992
 31. El-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW, Vogelstein B : WAF1, a potential mediator of p53 tumor suppression. *Cell* 75 : 817-825, 1993
 32. Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, Matsui K, Takei Y, Nakamura Y : A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. *Nature* 404 : 42-49, 2000
 33. Selvakumaran M, Lin HK, Miyashita T, Wang HG, Krajewski S, Reed JC, Hoffman B, Liebermann D : Immediate early up-regulation of bax expression by p53 but not TGF beta 1 : a paradigm for distinct apoptotic pathways. *Oncogene* 9 : 1791-1798, 1994
 34. Oda K, Arakawa H, Tanaka T, Matsuda K, Tanikawa C, Mori T, Nishimori H, Tamai K, Tokino T, Nakamura Y, Taya Y : p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. *Cell* 102 : 849-862, 2000
 35. Ziegler A, Jonason AS, Leffell DJ, Simon JA, Sharma HW, Kimmelman J, Remington L, Jacks T, Brash DE : Sunburn and p53 in the onset of skin cancer. *Nature* 372 : 773-6, 1994
 36. Nelson MA, Einspahr JG, Alberts DS, Balfour CA, Wymer JA, Welch KL, Salasche SJ, Bangert JL, Grogan TM, Bozzo PO : Analysis of the p53 gene in human precancerous actinic keratosis lesions and squamous cell cancers. *Cancer Lett* 85 : 23-29, 1994
 37. Urano Y, Asano T, Yoshimoto K, Iwahana H, Kubo Y, Kato S, Sasaki S, Takeuchi N, Uchida N, Nakanishi H, Arase S, Itakura M : Frequent p53 accumulation in the chronically sun-exposed epidermis and clonal expansion of p53 mutant cells in the epidermis adjacent to basal cell carcinoma. *J Invest Dermatol* 104 : 928-932, 1995
 38. Ren ZP, Hedrum A, Ponten F, Nister M, Ahmadian A, Lundeberg J, Uhlen M, Ponten J : Human epidermal cancer and accompanying precursors have identical p53 mutations different from p53 mutations in adjacent areas of clonally expanded non-neoplastic keratinocytes. *Oncogene* 12 : 765-773, 1996
 39. Quelle DE, Zindy F, Ashmun RA, Sherr CJ : Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. *Cell* 83 : 993-1000, 1995
 40. Stott FJ, Bates S, James MC, McConnell BB, Starborg M, Brookes S, Palmero I, Ryan K, Hara E, Vousden KH, Peters G : The alternative product from the human CDKN2A locus, p14 (ARF), participates in a regulatory feedback loop with p53 and MDM2. *EMBO J* 17 : 5001-14, 1998
 41. Bruce JL, Hurford RK Jr, Classon M, Koh J, Dyson N : Requirements for cell cycle arrest by p16^{INK4a}. *Mol Cell* 6 : 737-42, 2000
 42. Kubo Y, Urano Y, Matsumoto K, Ahsan K, Arase S : Mutations of the *INK4a* locus in squamous cell carcinomas of human skin. *Biochem Biophys Res Commun* 232 : 38-41, 1997
 43. Soufir N, Moles JP, Vilmer C, Moch C, Verola O, Rivet J, Tesniere A, Dubertret L, Basset-Seguain N : P16 UV mutations in human skin epithelial tumors. *Oncogene* 18 : 5477-5481, 1999
 44. Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelutz AJ : Both Rb/p16^{INK4a} inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* 396 : 84-88, 1998
 45. Knudson AG : Antioncogenes and human cancer. *Proc Natl Acad Sci USA* 90 : 10914-21, 1993
 46. Quinn AG, Sikkink S, Rees JL : Basal cell carcinomas and squamous cell carcinomas of human skin show distinct patterns of chromosome loss. *Cancer Res* 54 : 4756-4759, 1994
 47. Quinn AG, Campbell C, Healy E, Rees JL : Chromosome 9 allele loss occurs in both basal and squamous cell carcinomas of the skin. *J Invest Dermatol* 102 : 300-3, 1994
 48. Ahmadian A, Ren ZP, Williams C, Ponten F, Odeberg J, Ponten J, Uhlen M, Lundeberg J : Genetic instability in the 9q22.3 regions is a late event in the development of squamous cell carcinoma. *Oncogene* 17 : 1837-1843, 1998

49. Goudie DR, Yuille MA, Leversha MA, Furlong RA, Carter NP, Lush MJ, Affara NA, Ferguson-Smith MA : Multiple self-healing squamous epitheliomata (ESS1) mapped to chromosome 9q22-q31 in families with common ancestry. *Nat Genet* 3 : 165-9, 1993
50. Choate KA, Medalie DA, Morgan JR, Khavari PA : Corrective gene transfer in the human skin disorder lamellar ichthyosis. *Nat Med* 2 : 1263-1267, 1996
51. Kubo Y, Dajee M, Lazarov S, Tao S, Khavari P : Induction of human epidermal neoplasia resembling squamous cell carcinoma (SCC) by defined genetic elements Ras and CDK4. (Abstracts for the 62st annual meeting of the SID) *J Invest Dermatol* 117 : 472, 2001