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# Cytogenetics of Melanoma and Non-Melanoma Skin Cancer

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## Abstract

Cytogenetic analysis of melanoma and non-melanoma skin cancers has revealed recurrent aberrations, the frequency of which is reflective of malignant potential. Highly aberrant karyotypes are seen in melanoma, squamous cell carcinoma, solar keratosis and Merkel cell carcinoma with more stable karyotypes seen in basal cell carcinoma, keratoacanthoma, Bowen's disease, dermatofibrosarcoma protuberans and cutaneous lymphomas. Some aberrations were common amongst a number of skin cancer types including rearrangements and numerical abnormalities of chromosome 1, -3p, +3q, partial or entire trisomy 6, trisomy 7, +8q, -9p, +9q, partial or entire loss of chromosome 10, -17p, +17q and partial or entire gain of chromosome 20. Combination of cytogenetic analysis with other molecular genetic techniques has enabled the identification of not only aberrant chromosomal regions, but also the genes that contribute to a malignant phenotype. This review provides a comprehensive summary of the pertinent cytogenetic aberrations associated with a variety of melanoma and non-melanoma skin cancers.

**Abbreviations:** BCC, basal cell carcinoma; BD, Bowen's disease; CBCL, cutaneous B-cell lymphoma; CGH, comparative genomic hybridization; CMM, cutaneous malignant melanoma; CTCL, cutaneous T-cell lymphoma; DFSP, dermatofibrosarcoma protuberans; FISH, fluorescence in situ hybridization; FL, follicular lymphoma; KA, keratoacanthoma; LOH, loss of heterozygosity; MCC, Merkel cell carcinoma; MF, mycosis fungoides; NMSC, non-melanoma skin cancer; SCC, squamous cell carcinoma; SK, solar keratosis; SS, Sezary syndrome; UV, ultraviolet

## Introduction

Skin cancer represents an accumulation of genetic abnormalities, inherent and/or sporadic, that alter the cells in such a way that normal function is impaired and a tumor arises. Malignant melanoma originates from aberrant melanocytes located in the basal (innermost) layer of the epidermis, whereas non-melanoma skin cancer (NMSC) is due to abnormalities of other cell types. The two most common forms of NMSC are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), arising from keratinocytes in the basal and squamous ($2^{nd}$ innermost) layers, respectively. [1] Other NMSCs include those developing from keratinocytes: solar keratosis (SK), SCC in situ (Bowen's disease; BD), and keratoacanthoma (KA); [2] and those arising from other cells within the skin or associated with the skin, such as: eccrine carcinomas, apocrine and sebaceous gland carcinomas, Kaposi sarcoma, liposarcoma, malignant fibrous histiocytoma, cutaneous lymphoma, Merkel cell carcinoma (MCC), extramammary Paget's disease, leiomyosarcoma, epithelioid sarcoma, malignant schwannoma, malignant granular cell tumor and dermatofibrosarcoma protuberans (DFSP). [2]

Although the development of both melanocytic and non-melanocytic skin cancers is strongly reliant upon genetic factors, such as skin type, familial incidence (including inherited disorders), race and gender, it is also heavily influenced by environmental factors, most specifically ultraviolet (UV) radiation. [3, 4] UV radiation is known to induce DNA point mutations and small deletions. [5, 6] Also, Emri and colleagues detected the formation of micronuclei, cytogenetic indicators of chromosomal damage, in melanocytes and fibroblasts exposed to UVB radiation

and to a lesser extent in fibroblasts exposed to UVA radiation. [7] This study indicated that at physiological doses of UV radiation, gross chromosomal aberrations can be induced. [7]

Cytogenetic analysis is a powerful tool used in the identification of chromosomal aberrations with applications ranging from identification of pre-natal birth defects to detection and prognostic evaluation of diseases associated with sporadic or inherent karyotypic abnormalities. A wide variety of karyotypic abnormalities are associated with diseases, including changes in karyotypic number, size and structure. In particular, cytogenetic analysis has given invaluable insight into abnormalities associated with hematological malignancies and solid tumors. An important consideration in cytogenetic analysis of solid tumors is clonal derivation as tumors may be of monoclonal (tumors evolve from changes in a single cell) or polyclonal (tumors evolve from multiple cells, each with distinct mutations) origin. Thus, cytogenetic reports often refer to changes associated with different clonal populations and also to random mutations, which are not clonal changes. Particularly relevant to skin cancers is field cancerization, a process where a large epithelial area may have undergone pre-neoplastic change after prolonged mutagenic exposure resulting in multiple independent tumor foci. [8] As such, there is an increased risk of additional tumor development after the emergence of a tumor and consequently these tumors, although distinct entities, may share similar cytogenetic abnormalities. [8]

Aneuploidy, or abnormal DNA content, is a consequence of defects in the mitotic checkpoint and is the most common characteristic of solid tumors. [9] In fact, the frequency of aneuploidy in tumors suggests that it either contributes to or drives tumorigenesis, as well as indicating malignant potential. [9, 10] Aneuploidy is typically detected by flow cytometry or image analysis,

with these techniques showing high concordance (>90%) and comparisons with cytogenetic analysis also showing high concordance (>80%). [11, 12] DNA aneuploidy and tetraploidy in cancer cells has been associated with poor prognosis, advanced staging and poor histologic differentiation in a variety of solid tumors. [13, 14]

Direct analysis of tumor karyotypes allows the detection of various structural and copy number changes that may be a cause or consequence of tumorigenesis. Analyses of hematological malignancies have shown non-random, recurrent karyotypic abnormalities that are often highly specific to one or more cancer types. [15] In particular, the Philadelphia chromosome, arising from a reciprocal translocation, t(9;22)(q34;q11.2), was the first consistent abnormality identified in human cancer and some believe that it should be the defining characteristic of chronic myeloid leukemia. [15] The analysis of solid tumors has been more problematic due to the culture of cells, which may lead to differential cell type selection, subclone selection and cell bias. [16-18] Studies of NMSC cultures have shown fibroblastic rather than the expected epithelial growth pattern, potentially indicating preferential growth of contaminating stromal fibroblasts. [16] Analysis of eight cell lines derived from malignant melanoma demonstrated selection of a subclone and subsequent emergence of its own subclones in each of the lines such that no long-term culture was identical to its line of origin. [17] Additionally, cell culture can be biased toward selection of tumor cells that have a high mitotic index, therefore the culture may not represent the entire tumor specimen. [18] Each of these studies demonstrate inadequacy in some cytogenetic analyses of solid tumors, including skin cancers. As such, caution is warranted in the interpretation of results in studies involving cell culture of skin tumors, particularly long-term culture.

Comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) are fluorescence based molecular tools used for the detection of molecular aberrations. CGH involves hybridization of differentially labeled tumor and reference DNA to normal metaphase spreads to globally screen for gross (>20mb) copy number aberrations. [19] Imbalances are detected by changes in fluorescence values of the tumor DNA relative to the reference DNA and as such, it does not require the interpretation of complex tumor karyotypes or prior knowledge of aberrations for probe design. [19, 20] Although CGH only reveals relatively large numerical or unbalanced aberrations, it identifies previously unknown DNA copy number changes of regions that may harbor tumor suppressor genes or oncogenes associated with cancer development. FISH also allows the identification of copy number changes, both large (up to whole chromosomes) and small (as low as 1-200kb), as well as detecting translocations and inversions that are difficult to determine using standard karyotyping techniques [20-22]. As probes designed for FISH analysis are of a known, specific sequence, they can be hybridized to cells in interphase, making FISH a useful technique in the identification of aberrations in archival material such as frozen or paraffin embedded samples. [20]

Each technique used for cytogenetic analysis has both advantages and disadvantages, however a combination of these cytogenetic techniques in addition to other molecular genetic techniques can provide a comprehensive overview of abnormalities that drive tumorigenesis and may even predict outcome of the disease. Although not strictly a cytogenetic technique, loss of heterozygosity (LOH) is often used in conjunction with cytogenetic analysis to confirm loss of regions or genes involved in tumorigenesis. Using highly polymorphic markers, or specific genes, a comparison of alleles from normal and tumor DNA of an individual is made; a loss of

an allele in the tumor tissue identifies or confirms a deleted chromosomal region. [23] These regions of genetic loss may harbor one or more putative tumor suppressor genes pivotal in cancer development. Also, gene expression analysis can be useful in determining if aberrant regions correlate to transcriptional activity. Using cDNA microarrays, overexpression of several genes has been found to correlate with breakpoint or amplified regions, [24, 25] although others suggest this rate of association is quite low (3.8%). [26] The aim of this review is to present a comprehensive summary of cytogenetic and associated abnormalities involved in melanocytic and non-melanocytic skin cancer development.

## Melanocytic derived skin cancers and their precursors

Malignant melanoma is the most fatal of the skin cancers, arising from the malignant transformation of cells (melanocytes) found within the basal layer of the epidermis. [27, 28] Lifetime risk for melanoma has been estimated at 2.04% for men and 1.45% for women in the US, with rates as high as 8.33% and 5.88% in Queensland, Australia for men and women, respectively. [28, 29] Melanoma can be classified into two major groups based on location of the lesion; cutaneous (skin; CMM) and uveal (eye), both of which have numerous chromosomal abnormalities. [4] Melanoma stages I-IV are typically defined by level of invasion (Clark microstaging levels I-V) and tumor thickness (Breslow microstaging). [30-32] Melanoma stages I and II refer to primary lesions yet to metastasize, stage III refers to lesions that have invaded the regional lymph nodes and stage IV refers to lesions that have metastasized to distant sites. [30] Dysplastic nevi are considered a precursor to CMM and although most are biologically stable, their presence has been associated with 100% of familial and 60% of sporadic CMM cases. [33]

In a study of 34 nevi and 53 melanoma, aneuploidy was observed in 2.94% of nevi, 0% of level I-III melanoma, 34.48% of level IV melanoma and 100% of level V melanoma (Clark microstaging)and was associated lesion thickness (0% in lesions <0.76 and 83% in lesions >3.0mm). [34] Additionally, in a follow-up examination of a sub-group of patients 90% of aneuploid tumors showed recurrence, whereas only 17.39% of diploid tumors recurred; of the 15.09% of tumors that regressed during the study, all were diploid. [34] A study by Kheir and colleagues retrospectively examined 177 stage I cutaneous melanomas, including those in the previously mentioned study, and found aneuploidy to be highly predictive of both recurrence and

shorter disease-free survival. [35] Aneuploidy (as opposed to diploidy) was associated with increased thickness (8% of tumors <1.5mm; 39% of tumors ≥3.0mm), Clark's level (11% of tumors level I, II or III; 31% of tumors level IV or V), ulceration (11% in ulcerated tumors; 35% in non-ulcerated tumors), vertical growth (0% of tumors with no growth; 26% of tumors with vertical growth), cell type (17% of epithelioid tumors; 30% of other tumors) and location (12% of tumors of head, neck and trunk; 32% of tumors at extremities). [35]

Many studies have investigated karyotypic abnormalities associated with CMM and a recent comprehensive review of all previously published karyotypes outlined the identified recurrent aberrations. [4] Höglund and colleagues used the Mitelman Database of Chromosome Aberrations in Cancer [36] to identify recurrent imbalances, including deletions, additions, isochrome formation and somy changes associated with 92 cases of cutaneous melanoma. [4] The most common aberrations detected were -10 (59%), -6q10-q27 (42%), -9p10-p24, -21 (37%), +7, -16 (36%), -14, +1q24-q44, -4, -15 (33%), -5 (32%), -1p10-p36, -11q23-q25 (28%), -12q13-q24, +20 (27%), -17p, +18 (26%), -8p10-p23, +8q10-q24 (25%), -3 (24%), -22, -X (23%), +6p21-p25, -18 (22%), +3 (18%), -19 (17%), +9q22-q34 (15%), +19 (14%), +13, +17q10-q25 (12%), +2, +15, +21 and +22 (11%). [4] Two major karyotypic pathways were detected; the first involved +6p, -6q and possibly -16 as early cytogenetic changes and the second involved -3 and either +8q or -8p as early changes. [4] In addition to these imbalances, balanced translocations involving regions on 1q, 6q, 14q and 19p have been identified in a smaller percentage of cases. [36]

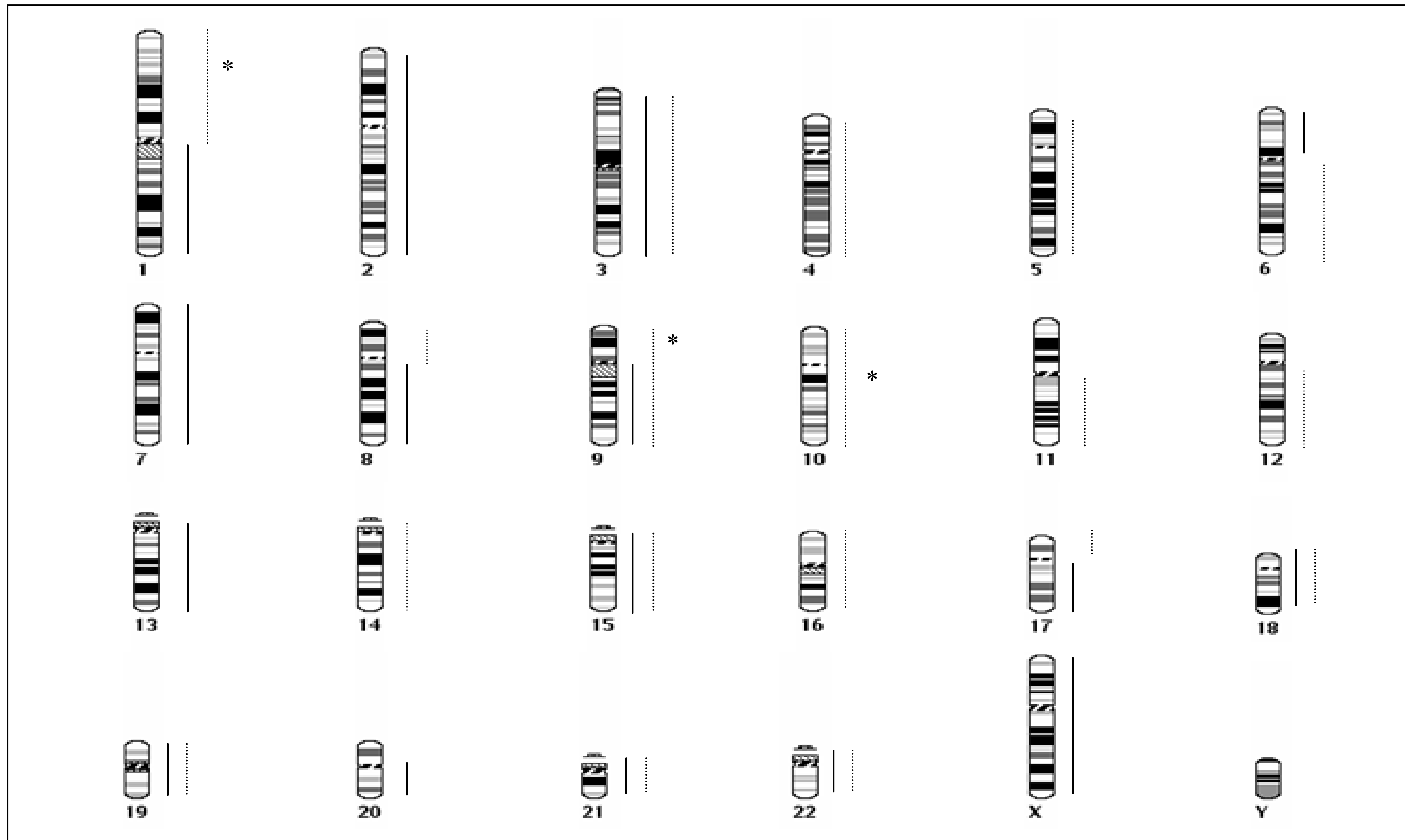
Some studies have investigated cytogenetic changes associated with dysplastic nevi, a precursor lesion to melanoma. One of these studies found loss of chromosome 9 in 2 of 4 dysplastic nevi,

suggesting this may be a primary event in the transformation of melanocytes. [37] Another study investigating three nevi from a single patient with a family history of melanoma found simple translocations in each of the nevi, including one with a 6q13 breakpoint (t(6;15)(q13;q21)), a region implicated in CMM. [36, 38] An investigation of eight benign nevi also revealed single occurrences of reciprocal translocations involving t(6;15), t(10;15), t(15;20) and t(4;5). [39] This demonstrates that even in benign lesions, chromosomal instability may have already begun and could potentially signify lesions that undergo malignant transformation.

Bastian, *et al*, analyzed 132 melanomas and 54 benign nevi by CGH and found that although 96.2% of melanomas exhibited copy number aberrations, only 13% of the nevi displayed aberrations. [40] Specifically, of the seven nevi that exhibited aberration, six of these showed a gain of the entire 11p arm, which was not found in any of the melanomas. [40] In the melanomas studied, regions of recurrent gain included 6p (37%), 1q (33%), 7p, 7q (32%), 8q (25%), 17q (24%) and 20q (22%), with regions of recurrent loss including 9p (64%), 9q, 10q (36%), 10p (30%), 6q (26%) and 11q (21%). [40] An earlier study on a smaller number of melanomas (16) also included analysis of metastatic tumors (12) and found similar aberrations as those listed above for both lesions. Additionally, gains of 5p, 5q21-q23, 10p and 18q and losses of 2p21-pter, 11q13-q23, 12q24.1-qter, 19q13.1-qter and 22qter were detected in metastatic lesions but not in primary lesions and losses involving 9p and 17 occurred at a higher frequency in metastatic tumors. [41] Specifically, investigation of primary and metastatic lesions excised from the same patient (4 patients) showed additional aberrations associated with metastases, although none were determined to be recurrent. [41]

FISH analysis of melanoma has verified aberrations of: extra copies (89%) and translocations (25%) of chromosome 20 (whole chromosome painting); [42] extra cyclin D copies in primary (47%) and metastatic (35%) lesions; [43] higher rates of trisomy seven in metastatic lesions (25%) compared to primary lesions (8%); [44] extra copies of c-myc in nodular (61%) and superficial spreading (27%) melanomas; [45] and copy number gains of 7 (40.9%), 6, 17 (27%), 9 and 10 (23%) as well as monosomies of 10 (55%), 9 (37%), 6 (27%), 17 (23%), 1 and 7 (18%) in malignant melanoma (14 primary and 8 metastatic). [46] LOH has been detected for at least one locus at 9p22 (31%), 10q11 (31%) and 1p36 (15%) in early stage CMM (13 cases), [47] and further analysis comparing benign and dysplastic nevi to CMM have shown higher frequency of 1p and 9p LOH in CMM (29% and 50% at most frequently lost loci) compared to dysplastic nevi (12% and 27% at corresponding loci) with complete absence of LOH in benign nevi. [48] Figure 1 summarizes recurrent aberrations (>10%) detected in CMM samples using a combination of karyotypic analysis, CGH and LOH studies.

**Figure 1: Summary of recurrent genomic aberrations in cutaneous malignant melanoma.**




| ______ Gain | ……... Loss | ● Breakpoint | * LOH |
|---|---|---|---|

**Keratinocytic derived skin cancers and their precursors**

Basal cell carcinomas are the most common form of NMSC, with an estimated lifetime risk between 28% and 33%. [49] Although they are locally invasive and highly destructive, BCCs rarely metastasize with an estimated metastatic potential of 0.0028-0.55%. [50] Squamous cell carcinoma is the second most common form of non-melanoma skin cancer and also the most metastatic, accounting for about 20% of all cutaneous malignancies with a lifetime risk estimated at 7-11%. [51, 52] The metastatic potential of SCC is highly variable from as low as 3.6% to 30% depending on the site and etiology of the lesion. [53] Solar keratosis (SK) is a lesion commonly described as a biomarker for both melanoma and non-melanoma skin cancer, with prevalence ranging from 11% to as high as 80%. [54, 55] They are known to undergo progression to SCC (0.1-10%) and it has been proposed that all SCC are derived from SK, implicating these lesions as the first recognizable stage of NMSC. [54, 56, 57] Additionally, up to 25% of lesions spontaneously regress. [55] SCC in situ, commonly known as Bowen's disease, is a lesion that infrequently progresses to SCC (about 2-5%) and has limited metastatic ability. [58, 59] Keratoacanthoma (KA) is arguably classified as either a distinct lesion or a subtype of SCC, with typical solitary lesions displaying a typical pattern of growth, maturation and spontaneous regression. [53] As SCC is considered to be the most aggressive form of NMSC, it might be expected that they exhibit a large degree of chromosomal instability. In turn, precursors of SCC or less aggressive forms of skin cancer such as SK, BD and KA and also BCC, would likely show less severe instability.

The incidence of DNA aneuploidy in BCC is fairly low (9-40%), which is indicative of its stability. [13, 60, 61] (remove 63) However, variability in aneuploidy rates has been seen in specific subgroups with rates of 80% for keratotic, 58% for metatypical, 24% for adenoid and 12% for

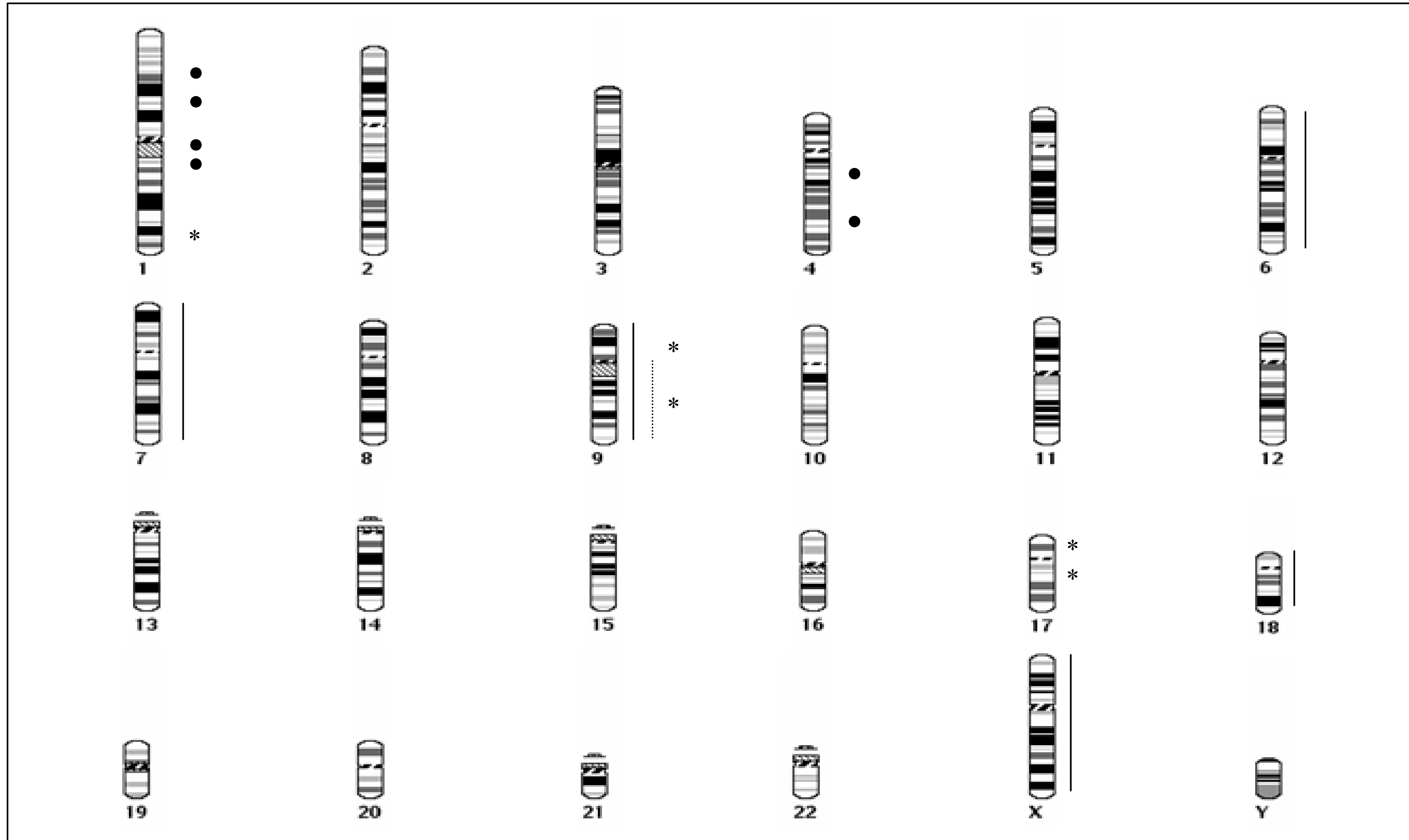
solid and cystic forms. [62] Aneuploidy rates are estimated to be higher in SCC (25-80%), concurring with the malignant spectrum. [13, 61, 63] A study by Pilch and colleagues also detected differences between well (46%) and moderately (75%) differentiated SCC. [63] Aneuploidy rates for SK, BD, and KA have been estimated at 69%, 89-92% and 4%, respectively. [63-65] Aneuploidy rates for SCC in situ (89-92%) are higher than those reported for both cutaneous SCC and SK, and as such Kawara and colleagues have suggested that DNA aneuploidy may not be a good prognostic marker of cutaneous SCC. [64, 65] However, aside from these discrepancies in BD data, aneuploidy rates in tumors appears to be indicative of malignant potential with rates for SCC similar to or higher than its precursor lesion, SK, and also higher than rates for most forms of BCC.

A number of recurrent numerical and structural alterations have been detected in BCC, although much of this has been done in short-term cultures, which allows for contamination of other cell types, subclone selection and cell bias. Using a chemically defined media for selection of epithelial cells, Jin and colleagues investigated 69 new and previously published short-term (5-10 days) BCC cultures and identified numerical aberrations of +18 (30%), +7, +X (17%) and +9 (14%) as well as rearrangements of 9q (24%) and breakpoints involving 1p32, 1p22, 1q11, 1q21, 4q21and 4q31 (10%). [66, 67] Less frequent breakpoints were also detected. Most of the cultures investigated had simple aberrant karyotypes consisting of only 1 to 3 aberrations, suggesting that these tumors are still relatively genetically stable compared to most other tumor types. [66, 67] Casalone and colleagues studied both direct preparations (24 hours; 73 samples) and short-term (10-28 days) cultures and showed inconsistencies in the aberrations detected in each of the techniques. [68] In direct preparations, the most recurrent and non-random change observed was +6

(in a small number of cases). [68] Confirmatory FISH analysis detected +6 in a further 8 (of 21) cases where trisomy six was not observed cytogenetically, although this aberration was not detected in any of the short-term cultures. [68] Similar to data found by Jin *et al*, a number of other studies have identified structural abnormalities, such as translocations and inversions involving 9q as a common event in BCCs. [16, 69, 70]

CGH analysis has also confirmed that there is a reasonable degree of genomic stability associated with BCC, finding that loss of genetic material is generally confined to 9q (33%), a region implicated in karyotypic analysis. [71] This loss was verified by LOH analysis, further defining the region to 9q22.3 in 53% of cases. [71] Regions of recurrent genetic gain were also detected using CGH analysis at 6p (47%), 6q, 9p (20%), 7 and X (13%). [71] The gain of 6p is also consistent with karyotypic and FISH analysis, which have identified trisomy six as a frequent aberration associated with BCC. [68] LOH studies in a Greek population have demonstrated losses of 9p21-p22 (55%), 17q21 (34%), and 17p13 (11%). [72] Quinn, *et al*, investigated LOH of BCC using a panel of microsatellite markers, finding loss at 9q (60%) and 1q (14%). [73] Other studies have also verified LOH at 9q22 in 46-60% of cases. [74, 75] A summary of recurrent aberrations (>10%) detected in BCC samples by karyotypic analysis, CGH and LOH can be seen in Figure 2.

**Figure 2: Summary of recurrent genomic aberrations in basal cell carcinoma.**




| _____ Gain | ……... Loss | ● Breakpoint | * LOH |
|---|---|---|---|

In a study of short-term cultures of 13 primary cutaneous SCC and review of 10 previously published cases, Jin and colleagues detected recurrent numerical gains of +7p (32%) and +8q (27%), and losses of -21 (41%), -8p (36%), -4p, -11p, -Y (32%), -13, -18q (27%), -10p, -X (23%) and -9p (18%). [8] They also detected various anomalies in the form of isochromes: i(1p10), i(1q10), i(5p10), i(8q10), i(9p10), i(9q10), (all <20%); with i(8q) and i(9q) believed to be early genetic events. [8] Casalone, *et al*, examined direct preparations of three primary cutaneous SCC finding aberrations that had not been previously considered recurrent in short-term cultures; these included -1, +6, +8, +9, +11, -14, +16 and +21. [68] However, as only three SCCs were investigated, these regions cannot be classified as recurrent. Most other cytogenetic studies have examined only small numbers of cultured SCCs or examined SCCs from Xeroderma Pigmentosum patients and have not revealed any new information. Available cytogenetic data on other keratinocytic tumors is somewhat limited. Short-term culture of three SKs and two cases of SK with SCC in situ has identified numerical changes of +7 and +20 (40%) and structural rearrangements involving chromosomes 1 (100%) and 4 (75%), however these numbers are too low to be considered informative. [76] Heim, *et al*, investigated short-term cultures from a single SCC in situ and although early passages revealed various aberrant clones, passages 7-11 were dominated by a single clone with a sole anomaly of t(12;17)(p13;q21). [77] Analysis of KA in two studies (1 lesion each) has identified 2p13 alterations in both lesions, although the type of aberration was different in the two lesions; no other anomalies were shared. [78, 79]

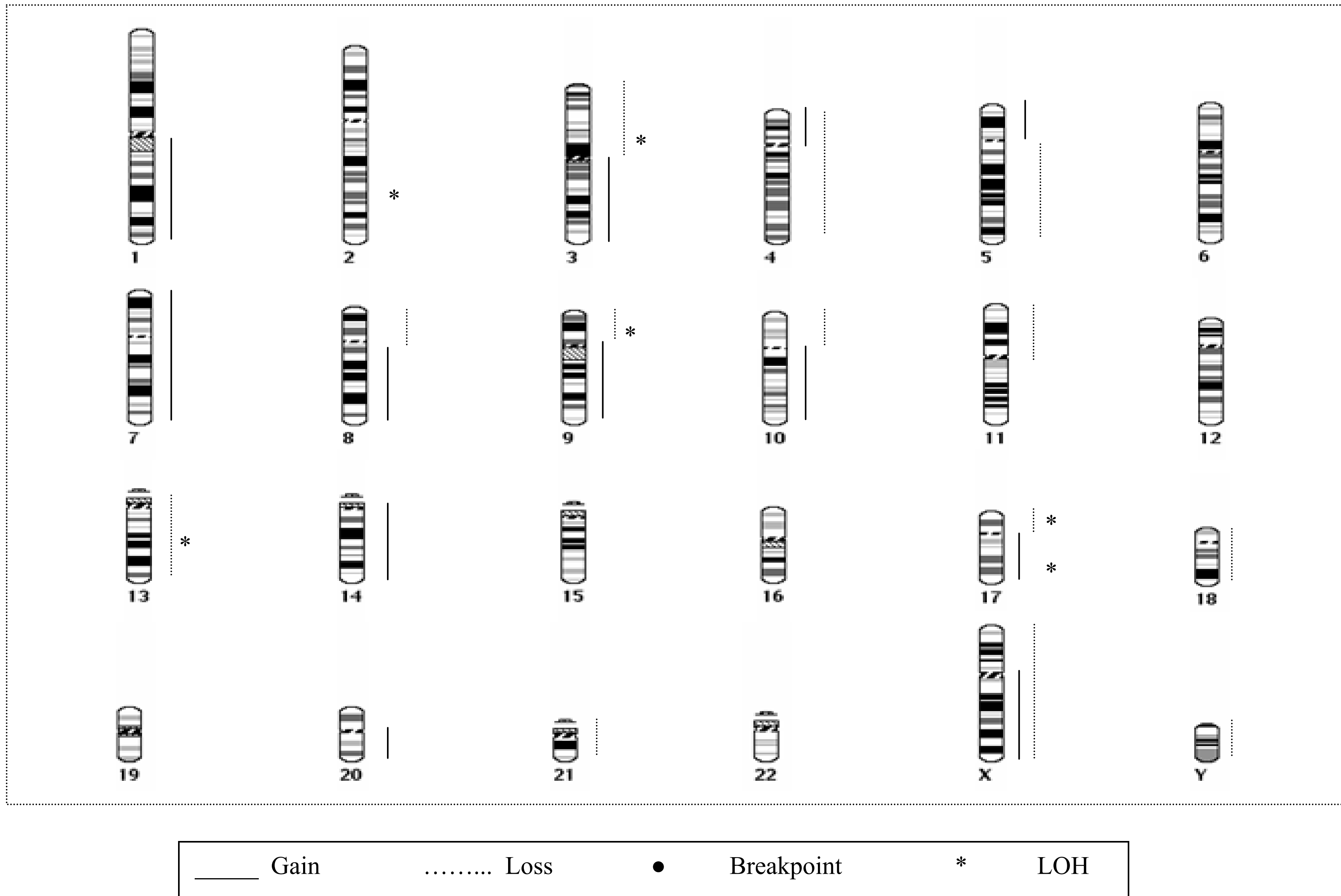
Early CGH studies have investigated single cases of SCC and their related recurrences and metastases and although they found many copy number aberrations, including anomalies unique to metastatic lesions, recurrent aberrations could not be defined. Our laboratory has investigated

SCCs and SKs finding a similar pattern of aberrations in the two lesions, supporting their close relationship. Aberrations in SCC included gains of 3q (47%), 17q (40%), 14q, Xq (33%), 4p, 8q (27%), 1q, 5p, 7q, 9q, 10q and 20q (20%), and losses at 3p (53%), 18q (47%), 17p (33%), 4q (27%), 5q, 8p, 11p, 13q and 18p (20%). [80] For SK, gains were seen at 3q, 4p, 17q (33%), 5p, 9q and 17p (25%) and losses at 9p, 13q (53%), 3p, 4q, 11p and 17p (25%). [80] The loss of 18q in 47% of SCCs was specific to this lesion (P = 0.04) and could likely harbor one or more genes that contribute to malignant progression. [80] Clausen and colleagues performed initial CGH analysis on KA lesions, mostly from immunosuppressed organ transplant recipients, and found copy number aberrations in 36% of KAs. Recurrent gains were detected at 8q (20%), 1p and 9q (16%) with losses at 3p, 9p, 19p (20%), and 19q (16%). [81] Their second study examined differences between KA and SCC and found gains of 1p, 14q, 16q, 20q and losses of 4p were significantly more frequent in SCC (P values ≤0.03), whereas a loss of 9p was significantly more frequent in KA (P = 0.04), supporting the theory that SCC and KA are distinct forms of NMSC. [82] CGH studies suggest that both SKs and SCCs are more genetically unstable than BCCs and KAs, showing a significantly higher number of aberrations indicative of a higher malignant potential.

Frequent LOH has been observed in SCC (including BD lesions) at 17q (43%), 13q (38%), 17p (34%), 9p (32%), 3p (26%) and 2q (20%). [73] A higher frequency of LOH has been observed in SK compared to SCC, with losses detected at 17p (64%), 13q (52%), 17q (46%), 9p (39%), 9q (22%) and 3p (31%), a pattern similar to that seen in SCC. [83] Individual analysis of a small number of BD has also revealed recurrent loss of *TP53* in a Korean population (27%). [84] Also, LOH of the region encoding *CDKN2A* occurs in 21% of SK and 46% of SCC, indicating a

possible role for *CDKN2A* inactivation in progression towards SCC. [85] LOH is rare in KA, with isolated losses detected at 9p, 9q, and 10q. [86] This low frequency of LOH further supports the theory that KA is a distinct lesion rather than a subtype of SCC. Figure 3 presents a summary of recurrent aberrations (>10%) detected in SCC samples by karyotypic analysis, CGH and LOH.

**Figure 3: Summary of recurrent genomic aberrations in squamous cell carcinoma.**



## Rare cancers of the skin

In addition to the most common forms of skin cancer, a number of rare benign and malignant lesions exist that arise from cells within the skin or are associated with tumor formation in the skin. Merkel cell carcinoma is a rare aggressive skin cancer arising from the neuroendocrine system. [87] Dermatofibrosarcoma protuberans is a rare cutaneous disease arising from spindle cells with a histology that may be fibroblastic, histiocytic or neural in origin.[88] Cutaneous T-cell lymphoma (CTCL) and cutaneous B-cell lymphoma (CBCL) arise from the lymphatic system. Subtypes include mycosis fungoides (MF) and Sezary syndrome (SS) for CTCL; and follicular lymphoma (FL), marginal zone B-cell lymphoma and large B-cell lymphoma for CBCL. [89, 90]

Even though these tumors are rare, a considerable amount of cytogenetic analysis has been performed on many of them. Table 1 summarizes some of the more pertinent studies that have been performed on these cutaneous lesions. Although other cytogenetic based analyses have been performed on additional rare skin tumors, Table 1 is generally limited to select tumors that have been investigated in multiple studies.

**Table 1: Common chromosomal aberrations associated with rare tumors of the skin.**

| Tumor | Major Findings | Number of cases |
|---|---|---|
| MCC | Karyotype: rearrangement of 1, -13 (67%), +11, -22 (33%) [91] | 6 cases |
| | CGH: +19q (63%), +19p (50%), +1p (54%), -3p (46%), +1q, +X (42%), +5p, +8q (38%), -10, +3q, -13q (33%), +20p (29%), +7p, -17p, +20q (25%), -5q, +6q, +7q, -8p, +13q, +18q (21%), -11q, +21 (17%); the average number of imbalances differed in patients surviving >24 months (6.6) and <24 months (11.2) [87] | 34 cases (24 patients) |
| | CGH: +6 (42%), +1q11-q31, +5p (32%), +1q32-qter (26%), +1p33-pter, +12, -13q13-q31 (21%), -4q (16%) [92] | 19 cases |
| | LOH: deletion of 10q23 (43%); unlikely involvement of *PTEN* [93] | 18 informative cases |
| | LOH: deletion of 1p35-36 (70%) [94] | 10 cases |
| DFSP | Karyotype/FISH: t(17;22)(q22;q13), which fuses *COL1A1* and *PDGFB*; often involves formation of ring chromosomes including sequences from these chromosomes (≈ 70%) [88] | Review of published cases |
| | CGH: +17q21-qter (100%), +22pter-q13 (82%), +5 (27%) [95]<br>CGH: +17q22-qter (83%), +22q13 (75%), 8q24.1-qter (25%) [96] | 11 cases; 12 cases |
| | CGH: +17q21-qter (100%), +22pter-q13 (70%), +5 (40%), +1 (40%), +12p, +12q23-qter, +21 (30%), +8, +20, -X (20%) [95] | 10 cases (fibrosarcomatous transformation) |
| | Array CGH (pooled analysis): +8q24.3, +17q21.33-qter, +22cen-q13.1 (recurrent) [88] | 10 cases |
| CTCL | Karyotype (SS subtype): structural aberrations affecting 10, 17 (28%), 1p (22%), 6q, 14q (17%)<br>CGH: -1p (38%), -17p (21%), +4/4q (18%), -10q/10, +18, -19 (15%), +17q/17 (12%)<br>FISH (SS subtype): rearrangements of 1p, 17p (33%), 10 (27%) [97] | 18 cases (SS subtype); 16 cases (MF subtype) |
| | LOH (SS subtype): deletion of 9p (46%), 17p (42%), 10q, 2p (14%)<br>LOH (MF subtype): deletion of 9p (16%), 10q (12%), 1p, 17p (10%) [89] | 15 cases (SS subtype); 51 cases (MF subtype) |
| CBCL | FISH: translocations involving *IGH* (52%), *BCL2* (41%) and *BCL6* (7%) [98] | 27 cases (FL subtype) |
| | FISH: translocations involving *IGH* (50%), *MYC* (43%), *BCL6* (36%) [99] | 14 cases (large B-cell subtype) |
| | CGH (secondary large CBCL, 5 cases): -17p (60%)<br>CGH (primary large CBCL, 9 cases): +2q, +7q, +12, +13, -17p, +18, -19<br>CGH (FL subtype): +3q, +4, +7q [100] | 14 cases (large B-cell subtype); 4 cases (FL subtype) |

## Implications of Cytogenetic Findings

Cytogenetic analysis is an extremely powerful tool to aid in the detection of chromosomal abnormalities involved in neoplastic development, and when combined with other molecular techniques, putative genes related to the progression and often outcome of cancer can be identified. Skin cancer results from the accumulation of genetic aberrations that arise within cells of the skin. Malignant melanoma is a particularly aggressive form of skin cancer with high probability of metastasis, whereas non-melanoma skin cancer is typically less aggressive, although it does show a wide spectrum of clinical outcomes. Additionally, rare skin cancers display a range of clinical behaviors. As genomic instability is a hallmark of cancer aggressiveness, cytogenetic analysis of skin cancer has proven invaluable in detecting changes associated with malignancy and on occasion can be predictive of clinical outcome.

Analysis of aneuploidy in skin cancer is a simple method to detect abnormal chromosomal content. In CMM aneuploidy rates can be up to 100% in advanced disease and associations have been made with various adverse clinical parameters, including advanced disease indicators, disease recurrence and shorter disease-free survival. Aneuploidy rates are fairly low in BCC, higher for SK and even higher for SCC, indicating increased rates with malignant potential. Supporting this is an exceptionally low aneuploidy rate for KA, which are well known for their regression. However, aneuploidy rates for BD are typically very high, which is not reflective of their low metastatic potential. Aside from this exception, aneuploidy in skin cancer appears to

associate with malignant potential. Additionally, highly aberrant karyotypes were associated with CMM, SCC, SK and MCC, each of these exhibiting more aggressive biologic behaviors.

There were a number of aberrations that were very common amongst the different forms of skin cancer, including: rearrangements and numerical abnormalities of 1 (CMM, BCC, SCC, SK, KA, MCC, DFSP, CTCL); -3p (SCC, SK, KA, MCC); +3q (SCC, SK, MCC, CBCL); trisomy of all or part of 6 (CMM, BCC, SCC, MCC); trisomy 7 (CMM, BCC, SCC, SK, MCC and 7q of CBCL); +8q (CMM, SCC, KA, MCC, DFSP); -9p (dysplastic nevi, CMM, BCC, SCC, SK, KA, CTLC); +9q (CMM, SCC, SK, KA); loss of part or all of 10 (CMM, SCC, MCC, CTCL); -17p (CMM, BCC, SCC, SK, BD, MCC, CTCL, CBCL); +17q (CMM, SCC, SK, DFSP); and gain of all or part of 20 (CMM, SCC, SK, MCC, DFSP). Certain aberrations were contradictory in some cancers, showing amplifications and deletions with similar frequencies in the same region. Such examples include chromosomes 9q, 15, 18, 19, 21 and 22 in melanoma and 4q and 9q in SK. Such regions could be indicative of genomic instability and may not represent aberrations associated with malignancy, or may contain both tumor suppressor genes and oncogenes related to tumor development and progression. Studies have shown that amplified regions contain genes that are both over- and under-expressed, thus gross chromosomal aberrations do not reflect all changes at the gene level. [26]

However, identification of commonly aberrant regions in skin cancer has led to the speculation and even identification of putative tumor suppressor genes and oncogenes

involved in malignant progression. Of particular importance is loss of 9p, which occurs in various skin cancers with some studies implicating a higher rate of 9p21-p22 loss in more progressive stages of disease including metastatic melanoma versus CMM versus dysplastic nevi [41, 48] and SCC versus SK. [85] This region harbors the *CDKN2A* tumor suppressor gene, a known susceptibility gene for hereditary forms of melanoma, and it seems likely that loss of function of this gene is associated with malignant progression. [33, 85] However, various studies have been unable to find mutations in *CDKN2A* associated with all cases of loss and some studies have even indicated that there are two additional regions of interest that harbor tumor suppressor genes. [28, 72] Other potential tumor suppressor genes and oncogenes include: *FHIT* (3p14.2), loss associated with MCC and lung cancer development; [87] *LAZ3* and *BCL6* (3q26-q27); [87] *E2F3* (6p22.3), over-expressed in retinoblastoma and bladder cancer; [101] *GPNMB* (7p15), over-expressed in melanoma; [25] *CDK6* (7q21) and *NRCAM* (7q31), over-expressed I melanoma; [25] *MYCC* (8q24), amplified in lung cancer and over-expressed in uveal melanoma; [87] *PTEN* (10q23.3), loss of function mutations associated with Cowden disease, an autosomal dominant cancer-predisposition syndrome; [102] *P53* (17p13), present in about half of all human cancers; [72] *E2F1* (20q11), over-expressed in melanoma. [25] Additionally, many other tumor suppressor genes and oncogenes exist in these regions, and novel genes responsible for cancer development may yet to be discovered.

Cytogenetic analysis has also implicated different regions in specific skin cancers, with some of the more pertinent aberrations following. Loss of 18q was found to be significantly higher in SCC compared to SK and may harbor genes responsible for

transformation to a more malignant phenotype. 18q21 harbors a number of tumor suppressor genes, with *Smad2* or *Smad4* being likely candidates as they have been implicated in malignant progression in other diseases. [103, 104] Analysis has implicated 9q22 in sporadic BCC development, a region which contains the *PTCH* tumor suppressor gene. *PTCH* functions to protect epithelial cells against a variety of genetic hits and had originally been identified in Gorlin's syndrome, an autosomal dominant disorder associated with propensity for BCC development. [23, 71] Various cytogenetic techniques have now implicated the same region in sporadic BCC development. Translocation involving 17q22 and 22q13 is a hallmark of DFSP lesions, often involving the formation of supernumerary rings. This translocation creates a characteristic gene fusion between *COL1A1* and *PDGFB*, resulting in aberrant expression of *PDGFB*. [88]

In summary, cytogenetic analysis of melanoma and non-melanoma skin cancer has revealed a number of recurrent abnormalities. It is interesting to note that a large number of aberrations are common amongst different skin cancers, although whether the genes associated with these abnormalities are common is yet to be determined. Because much of cytogenetic analysis focuses on large aberrations, implicated regions may not always be responsible for malignant progression but may be a result of the genomic instability associated with cancer development. However, it has become clear that a number of regions implicated by cytogenetic analysis harbor tumor suppressor genes and oncogenes involved in tumorigenesis. LOH, mutational and methylation studies may aid in the identification of putative cancer related genes and gene expression analysis can give direct evidence of transcriptional activity associated with implicated genes. A

combination of cytogenetic analysis with other molecular techniques has implicated a number of chromosomal regions and associated genes in melanoma and non-melanoma skin cancer development and will continue to advance our knowledge in this area.

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