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Revealing the genomic heterogeneity of melanoma

The melanoma genome possesses numerous recurrent chromosomal rearrangements, and embedded within this complexity are clues critical to disease pathogenesis and response to therapy. High-resolution genome-wide DNA copy number approaches, in conjunction with gene-specific mutational analyses, appear poised to define keystone molecular events, provide more accurate classification schemes, and set the stage for the design of rational therapies that may finally have an impact on survival of this deadly disease.

The rapid rise in melanoma incidence and the high lethality associated with advanced disease (reviewed in Thompson et al., 2005) has motivated efforts to define the genetic and environmental factors driving melanoma genesis and progression. It is generally accepted that melanoma risk is modulated by skin pigmentation patterns, such as those linked to *MC1R* polymorphisms (Palmer et al., 2000), and early exposure to ultraviolet (UV) light (reviewed in Thompson et al., 2005). Stereotypical genetic lesions in melanoma include disruption of the *CDKN2A* familial melanoma locus that encodes for INK4A and ARF; activation of MAPK pathway components, commonly at the levels of BRAF and NRAS; and activation of the PI3K-AKT pathway through loss of PTEN (reviewed in Chudnovsky et al., 2005; Gray-Schopfer et al., 2005). Beyond these well-known and validated genetic events, genome-wide high-resolution technologies have been used to scan the highly complex melanoma genome, revealing the existence of additional genetic elements governing disease genesis and progression (Garraway et al., 2005; Curtin et al., 2005; O.K. and L.C., unpublished data). These data show that the life history of melanoma is shaped by extensive chromosomal rearrangements, particularly recurrent chromosomal gains/amplifications and losses/deletions. That these copy number alterations carry pathogenetic significance has been substantiated in a recent integrated genomics approach that has identified *MITF* as a lineage survival oncogene amplified in melanoma (Garraway et al., 2005). With increasing resolution of array CGH platforms for mapping chromosomal alterations and advances in expression and sequencing technologies, it is anticipated that the discovery of novel melanoma relevant genes will accelerate dramatically in the near future.

High-resolution charting of recurrent copy number aberrations by array CGH will also provide the basis for molecular classification that, when combined with clinical information, will define genotype-phenotype correlation and biomarkers that can enhance existing staging systems for patient stratification. For example, correlating melanomas arising in different anatomical sites with different UV exposure patterns to distinct genomic signatures should lead to an understanding of the genetic modulators and targets of UV's mutagenic actions in this cancer. In the November 17 issue of *The New England Journal of Medicine*, Curtin et al. (2005) took an important first step in defining such gene-environment interactions in melanoma.

In this study, Curtin and colleagues conducted a genome-wide analysis of DNA copy number and mutational analysis of *BRAF* and *NRAS* in 126 melanomas from individuals with varying UV exposure histories (Curtin et al., 2005). Distinctive patterns of genomic alterations as well as differences in frequencies of *BRAF* and *NRAS* mutations were observed among the four groups of melanomas examined. Genomic instability was most prominent in melanomas arising on skin protected from direct UV light. Specifically, acral melanomas of the palms and soles and mucosal melanomas exhibited high numbers of whole-genome gains and losses, intrachromosomal copy number changes, and focal amplifications (Figure 1). On the other hand, amplifications and deletions were infrequent in melanomas arising on skin with chronic sun-induced damage (as defined by evidence of solar elastosis on histology) and those from skin with intermittent UV exposure but without chronic damage (Figure 1). There were differences not only in the levels of genomic instability for the four melanoma subgroups, but also in their patterns of chromosomal gains and losses (Figure 1). Genomic classification was able to classify acral and mucosal melanomas with 89% accuracy. It was also possible to distinguish between melanomas from skin exhibiting signs of chronic sun-induced damage and those from skin without signs of damage with 84% accuracy. That genomic signatures capable of classifying melanoma from different anatomic sites can be defined is a definitive proof that melanoma is a genetically heterogeneous disease.

In addition to their genomic patterns, the mutational spectrum was different between melanomas from sun-exposed and sun-protected skin (Figure 1). In particular, this study extended the group's earlier findings that melanomas from chronically sun-exposed and non-sun-exposed skin differed significantly in the mutation frequency of *BRAF* (Maldonado et al., 2003). In all melanoma groups, *BRAF* and *NRAS* mutations were found to be mutually exclusive. The presence of activating *BRAF* mutations inversely correlated with copy number gains of *CCND1*, and both events were associated with higher levels of CCND1 protein expression. Amplification of *CDK4*, encoding a CCND1 binding partner, was commonly seen in

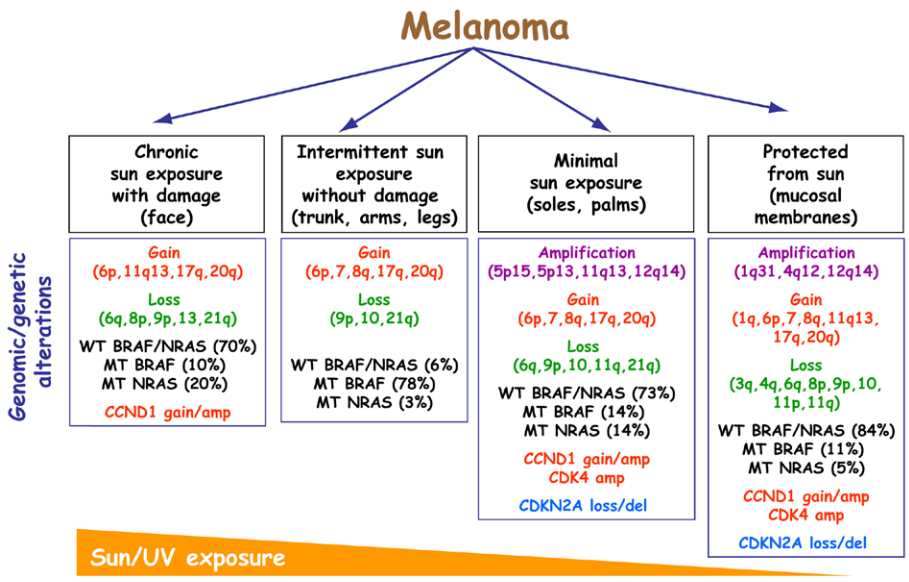

Figure 1. Summary of genetic alterations in melanomas from patients with varying degrees of sun exposure as reported by Curtin et al.

Melanomas from skin with evidence of chronic sun damage, intermittent sun exposure without evidence of damage, and no significant exposure exhibit distinct genomic patterns of gain/amplification and loss/deletion and different *BRAF/NRAS* mutational spectrums. *BRAF* and *NRAS* mutations were mutually exclusive, as were *BRAF/NRAS* activating mutations and increased copies of *CCND1* or *CDK4*. Gain/amplification of *CDK4* was more commonly seen in melanomas from protected skin than in melanoma from sun-exposed skin, as was deletion/loss of the *CDNK2A* locus, which was observed exclusively in samples without *CDK4* amplification. UV, ultraviolet light; WT, wild-type; MT, mutant; amp, amplification.

acral and mucosal melanomas but not observed in tumors with activating *BRAF* or *NRAS* mutations or *CCND1* copy number gains. Furthermore, losses/deletions of the melanoma suppressor *CDKN2A* locus, whose protein product antagonizes CCND1-CDK4 interaction, were also more commonly detected in mucosal and acral melanomas, but only in samples without *CDK4* amplification. Activation of the PI3K pathway was evidenced by increased levels of phosphorylated AKT protein in melanomas with concurrent *BRAF* mutations and chromosome 10 losses that encompassed the *PTEN* tumor suppressor, as well as in specimens with *NRAS* mutations alone, which is in line with the previous finding that *NRAS* mutations could result in dual activation of the MAPK and PI3K pathways (Tsao et al., 2000; Curtin et al., 2005).

While the differences between melanoma from sun-protected skin (acral and mucosal sites) cannot be attributed to UV, the distinct genomic profiles between melanomas arisen on chronically versus intermittently sun-exposed sites may provide new insights in defining complex gene-environment interactions that underlie disease pathogenesis. For instance, the study reports that melanomas from sun-damaged skin are typically wild-type for *BRAF* and often arise in conjunction with solar keratoses and other UV-related lesions in older individuals with high cumulative UV dose. In comparison, melanomas from skin intermittently exposed to UV harbor a high frequency of *BRAF* mutations and are often diagnosed in younger individuals who have larger numbers of moles. These contrasting characteristics have suggested to the authors that a potential difference in genetic susceptibility to UV exposure and melanoma exists in certain Caucasian populations (Curtin et al., 2005).

The recognition of the genomic heterogeneity of different types of melanoma has important clinical and therapeutic implications, as it has a direct impact on design and development of prevention and therapeutic approaches. For example, since activation of MAPK (BRAF/NRAS) and the PI3K pathways are important in the subgroup of melanoma from skin with intermittent UV exposure, which is the most common form of the disease, BRAF may provide a logical target for therapeutic intervention (reviewed in Tuveson et al., 2003). Conversely, in the case of melanomas from chronically sun-damaged skin or from UV-protected sites, which typically do not acquire *BRAF* mutations but rather amass higher copy numbers of *CCND1* or *CDK4*, a therapeutic approach involving CDK inhibitors might yield a positive response (Curtin et al., 2005). In addition, the definition of subtype-specific genomic and genetic signatures will serve as the entry point for discovery of novel melanoma genes that may serve as future therapeutic targets and may assist in the construction of refined preclinical models. As reported in this study, gain/amplification and loss/deletion appeared to encompass large chromosomal regions. Therefore, much work remains, including the examination of melanomas of similar and distinct etiologies and their metastatic counterparts utilizing high-resolution oligonucleotide or tiling BAC array platforms to identify a limited number of copy number-altered genes that can be enlisted into a functional validation pipeline.

Accurate diagnosis, prognosis, stratification, and management of melanoma patients will likely require integration of a multitude of biomarkers on epidemiological (e.g., UV exposure history), predisposition genetic (e.g., *MC1R* polymorphism or *CDKN2A* mutation), clinical (e.g., age, gender, or site), and histopathological (e.g., Breslow thickness, tumor-infiltrating lymphocytes) levels in addition to the molecular signatures. On this note, it is clear that chromosomal alterations and mutational analysis of key melanoma genes do not present a complete molecular view of the melanoma genome. Epigenetic modifications, transcriptome profiles of both coding and noncoding RNAs, and the tumor and/or serum proteomes will be required to generate a more comprehensive picture of the factors driving melanoma and other cancers in general. What Curtin and colleagues reported in this study represents a proof-of-principle illustration of the importance and power of systematic genome-wide characterization of clinically annotated tumor specimens.

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