Chapter 12

THE ROLE OF KISS1 IN MELANOMA METASTASIS SUPPRESSION

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Abstract
Among neoplasms, the severity and relevance of metastasis is perhaps most striking in the case of melanoma where surgical resection would conduct cure if not for the subsequent complications of distant metastatic foci. Compounding this notion are statistics revealing that the number of cases of malignant melanoma have doubled each of the last four decades (1) and autopsies of patients presenting with melanoma reveal lung invasion in approximately ninety percent of cases (2). Thus, a clear understanding of the mechanisms governing melanoma metastasis and the role of metastasis suppressor genes in controlling this cascade is essential. The KiSS1 metastasis suppressor gene, identified and functional in melanoma models, provides us with just such an opportunity. As we endeavor to elucidate the role of KiSS1, an understanding of melanoma metastasis suppression mediated by chromosome 6, which provided the platform for the discovery of KiSS1, similarly proffers important clues to understanding the mechanism of KiSS1 metastasis suppression.

COMPLETE METASTASIS SUPPRESSION BY CHROMOSOME 6

Of the relatively few models of metastasis suppression in the literature, arguably the most penetrant, is the impact of human chromosome 6 within the highly metastatic human melanoma cell line C8161. In this model, metastasis suppression is complete. An amelanotic human melanoma cell line derived from an abdominal wall metastasis, C8161, exhibits reproducible metastatic potential from orthotopic (subcutaneous or intradermal) and intravenous injections in athymic mice, generating an average of 100 lung metastases per mouse following tail vein injection (3). Introduction of an intact neomycin tagged human chromosome 6 into C8161 by microcell-mediated chromosome transfer (MMCT) produced a series of hybrid clones (designated neo6/C8161) which, with the exception of one clone, exhibited unchanged in vitro growth rates (4). In vivo, despite a slight latency in tumor formation and retarded growth rates, the incidence of tumor formation in the neo6/C8161 hybrids was unchanged. However, the cells were no longer metastatic. neo6/C8161 cells introduced either
by i. v. or s.c./i.d. injection failed to produce any macroscopic metastases and when mice in spontaneous metastasis assays were retained up to 30 weeks following tumor excision to compensate for the reduced growth rate of the hybrids, still no metastases were evident. While Welch et al. demonstrated this suppression with three distinct neo6/C8161 clones in the original communication (4), Miele et al. recently expanded the evidence showing complete suppression with four additional clones in both spontaneous and experimental assays (5,6).

The impact of chromosome 6 is not limited solely to C8161. Introduction of chromosome 6 into the metastatic melanoma cell line, MelJuSo, significantly inhibited the average number of lung metastases obtained in both the spontaneous and experimental metastasis assays (5). Tumorigenicity and in vivo growth rates of the neo6/MelJuSo clones were indistinguishable from the metastatic parental line and exhibited similar vascular invasion.

Nevertheless, the inherent genetic deficiencies of melanoma cell lines and the influences of chromosome 6 cannot be over-generalized. Tumorigenicity, not merely metastasis, was suppressed upon chromosome 6 introduction into the tumorigenic (but non-metastatic) human melanoma cell lines UACC-903 and UACC-091 (7).

DISCOVERY OF KISS1

The clear metastasis suppression mediated by chromosome 6 is consistent with the hypothesis that a metastasis-suppressor locus resides on this chromosome. Based on this idea, Lee and Welch utilized subtractive hybridization and differential display to identify differentially expressed genes, upregulated in neo6/C8161 and neo6/MelJuSo hybrids (8). In all, seven differentially expressed genes were isolated, including sequences bearing homology with rat nucleophosmin B23, transcription factor AP-2A, high-mobility group protein HMG-I(Y) and a partially sequenced fragment, 16A7. The three remaining novel clones exhibited no significant homology to DNA sequences known at that time. Among these, two clones – designated KiSS1 and KiSS16 – displayed a qualitative difference in expression between the metastatic parent and non-metastatic hybrids and were ideal subjects for further analysis.

Expression of the ~1.0 kb Kiss1 transcript was undetectable in the metastatic parental C8161 pool or subclones by northern analysis, but was present in all chromosome 6 hybrid clones (9). This qualitative difference sharply contrasted to the other differentially expressed clones which were often detectable in the metastatic cell lines but exhibited quantitative upregulation between 1.9- and 36-fold in either the C8161 or MelJuSo hybrids (8).