Case Report
Comparative genomic hybridization in a case of melanoma that loses expression of S100, HMB45, Melan A and tyrosinase in metastasis

Ruifeng Guo1, Xianfu Wang2, Jie Chen1, Elizabeth Gillies1, Kar-Ming Fung1, Shibo Li2, Lewis A Hassell1

1Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma city, OK, USA; 2Department of Pediatrics, Genetics Laboratory, University of Oklahoma Health Sciences Center, Oklahoma city, OK, USA

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Abstract: We recently reported three cases of metastatic melanoma that does not express S100, HMB45, Melan A and Tyrosinase. A concurrent cutaneous scalp primary melanoma was identified later in one of the cases, which showed strong expression of these markers. The difference in immunophenotype between the primary melanoma and its metastasis in the parotid gland in this case raised the question of the biological significance of the expression of these markers and metastatic potential. To address this question, we utilized microarray comparative genomic hybridization (aCGH) to compare the cytogenetic features between the primary and metastatic melanoma. We observed chromosomal gains including 6p, entire chromosome 7, and 8q11.1-q24.3 in both primary and metastatic tumors. However, the metastatic lesion showed unique additional copy of chromosomal 7q, and loss of chromosome 9p24.3-q13 and chromosome 4, which included Melan A encoding gene region in 9p24.1. The above findings suggest the unique cytogenetic changes in the parotid lesion are most likely related to the metastatic behavior, as well as responsible for loss of multiple melanocytic marker expression in the metastatic melanoma for this case.

Keywords: Melanoma, metastasis, cytogenetics, comparative genomic hybridization

Introduction

We recently reported three cases of metastatic melanoma from parotid gland, cheek, and right arm subcutaneous tissue respectively initially presenting as high grade tumor mimicking sarcoma accompanied by absent expression of S100, HMB45, Melan A and Tyrosinase [1]. Micro-ophthalmia transcription factor (MiTF) was the only marker indicative of their melanoma lineage. Further investigation revealed that these cases either had a remote previous history of primary melanoma, or concurrent/subsequent identification of a primary cutaneous melanoma that was not initially identified. However, one of these tumors, the primary tumor of the metastasis to the parotid gland, was strongly positive for S100 and pan-melanoma cocktail, which includes HMB45, Melan A and Tyrosinase [1]. This intriguing phenomenon has never been reported before and it raised the question as to whether the parotid lesion was truly a metastatic melanoma from the primary cutaneous site with alteration of immunophenotype, or a coincident independent melanoma with MITF expression but negative for other markers. We also hypothesized that the loss of these markers may reflect a genomic change tied to the metastatic potential. We addressed these two questions by comparing the genomic features of the cutaneous and parotid tumor by microarray comparative genomic hybridization (aCGH).

Case description

The detailed clinical and histopathologic features of the case have been previously described [1]. Briefly the patient initially presented with a histologically aggressive appearing (Figure 1A) necrotic parotid gland tumor that was negative for S100 and Pan-Melanoma cocktail (Figure 1B and 1C), but positive for MITF [1]. Further examination revealed a cutaneous nodular melanoma on the temporal scalp (Figure 1D) which showed positive staining for...
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S100 and Pan-Melanoma cocktail (Figure 1E and 1F). The parotid mass was considered to be a metastatic melanoma with loss of multiple melanocytic markers based on clinical and anatomic features. This consideration, however, could be challenged by arguing that the differing immunophenotypes imply separate neoplasms.

CGH analysis

To elucidate the molecular mechanism underlying the observed phenomenon, we applied high resolution aCGH [2] to both the primary cutaneous melanoma and the parotid melanoma and compared their cytogenetic features. The primary cutaneous melanoma showed multiple chromosomal gains, including 6p, almost entire chromosome 7, and 8q11.1-q24.3 (Figure 2A and 2C), which contain multiple important oncogenes, such as Myc (8q 24.21), RREB1 (6p25), epidermal growth factor receptor (EGFR) (7p12), and BRAF (7q34). The amplifications of the above loci have been well reported in melanoma. Interestingly, these changes are also present in the metastatic parotid lesion with generally stronger signals represented by more upwardly shifted dots, consistent with same genetic origin as the primary cutaneous melanoma (Figure 2A and 2C). It is noted that although the gain of chromosome 7q with BRAF gene is present in both primary and metastatic lesions, the copy number is evidently doubled in the metastatic tumor compared to the primary melanoma (Figure 2B).

The metastatic parotid lesion showed additional changes including loss of entire chromosome 4 and chromosome 9p24.3-q13, which were not present in the primary melanoma (Figure 2D and 2E). The most noteworthy tumor suppressor gene in these regions is CDKN2A (9p21), which encodes two splice variants, P16 and P14 ARF, both key regulators of the cell cycle. Deletion of partial or whole chromosome 4 has been reported in many types of malignancies including metastatic melanoma, therefore it has also been considered possessing critical tumor suppressor genes, despite the fact that their sequences and structures have not been clearly elucidated. The above findings are also summarized in Table 1.

The genes encoding S100 proteins, gp100 (antigen for HMB45) and tyrosinase are not affected in either primary or metastatic melanoma. However, the gene encoding melan A (Mart-1) is located at 9p24.1, which is deleted in the parotid metastatic lesion, which probably can explain the loss of expression of Melan A.
Discussion

We previously reported three cases of metastatic melanoma which lost expression of S100, Melan A, HMB45 and Tyrosinase but did express MiTF as the only lineage marker for melanoma [1]. A concurrent primary scalp melanoma was identified later in one case, which interestingly retained the expression of the above melanocytic markers. Although there were reports showing that loss of single melanocytic marker positivity could be observed in a small portion of metastatic melanoma, simultaneous negative staining for the above markers has never been described in the English literature.

The explanation for this phenomenon is intriguing. Additional questions are raised concerning the possible association between the loss of marker expression and the potential for metastasis. To elucidate these underlying mechanisms, we utilized high resolution microarray CGH to explore the cytogenetic changes in the case with both primary scalp melanoma and parotid metastatic melanoma. As expected, we observed typical melanoma CGH patterns for both primary and metastatic lesions, including chromosomal gains of 6p (RREB1), entire 7 (7q with BRAF), and 8q (Myc) [3-15]. Loss of 9p24.3-q13 (CDKN2A) [8, 11, 16-18] identified in solely the parotid metastatic lesion is also well reported in melanomas. These findings further confirmed our pathological diagnosis of metastatic melanoma in the parotid gland with unusual immunophenotype.

Despite the above, several novel observations arise from our CGH data. First of all, the metastatic lesion showed additional chromosomal abnormalities beyond those in the primary melanoma which is not entirely unexpected that metastatic tumor undergoes more genotypic changes in the process of metastasis, these additional changes are particularly interesting and may confer important information closely related to metastatic potential or processes. In this case the unique loss of chromosome 9p in the metastatic lesion has been reported as an important genomic change for cytogenetic identification of melanoma [8, 11, 16-18], but no one has studied its potential role in promoting melanoma metastasis as suggested by our result. Another important observation from this study is the loss of entire chromosome 4 in the metastatic lesion, which has been observed in metastatic lung [19-22], breast [23-26] and cervical cancers [20, 27].
Interestingly, an experimental model showed that losses of chromosomes 4 and 9p were associated with melanoma cell line liver metastasis in immunosuppressed mice, which is consistent with our observation in the clinical samples [28]. However a thorough study of its role in melanoma metastasis has not been conducted despite occasional observational reports [29]. Based on our observations, a larger scale clinical study to investigate the roles of the above chromosomal losses in melanoma metastasis should be highly informative.

The second interesting and important finding is the further amplification of chromosome 7q in the metastatic lesion [4, 7, 16]. BRAF gene located in this region has been identified as a major oncogene for cancer development through abnormal constitutive activation of mitosis activating protein kinase (MAPK) cascade, which is the major signaling pathway for cell growth and proliferation [8, 30-33]. Specifically it is considered the main driving force for melanocytic proliferation and present in more than 50% of benign melanocytic nevi and cutaneous melanomas [8, 30-33]. Newly developed specific BRAF kinase inhibitor has shown efficacy in prolonging survival in advanced melanoma patients who possess BRAF gene mutations leading to aberrant activation of this kinase [34-36]. In our case the amplification of BRAF gene locus was revealed, while the sequencing analysis still showed wildtype genotype of BRAF. This finding suggests that lack of BRAF mutation does not necessarily mean absence of BRAF associated MAPK pathway activation, and amplification of this wildtype gene can function as an alternative mechanism for melanomagenesis. Besides this mechanism, the additional copy of chromosome 7q in the metastatic lesion also raises the possibility that further accumulation of BRAF gene products or other previously unclassified gene products encoded in this region serves as an initial mechanism for melanoma metastasis. Further experimental and clinical studies of this aspect will be of great significance.

Finally, it is still intriguing that the metastatic lesion loses expression of all four melanocytic markers, leaving only MITF positivity. In our search through the genome database provided by the National Center of Biotechnology Information, we have found that the coding gene for Melan A/Mart-1 is normally located in chromosome 9p24.1, which is lost in the parotid metastatic melanoma. However, the coding genes for S100 protein family, gp100 (antigen recognized by HMB45 antibody) and tyrosinase are not located in any of the affected chromosomal regions identified in the metastatic lesion. Besides this, there is also a strong possibility that the mechanism for loss of marker expression is connected to the mechanism of metastasis, and the answer very likely resides in the two deleted chromosomal regions in the metastatic lesion. Further molecular biological studies in these two regions will provide critical information in unraveling the puzzle encountered here.

Disclosure of conflict of interest

None.

Address correspondence to: Lewis A Hassell, Department of Pathology, University of Oklahoma Health Sciences Center, BMSB 451, 940 Stanton L Young Blvd, Oklahoma City, OK 73104, USA. E-mail: lewis-hassell@ouhsc.edu

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