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[PDF (216K)] [References]

Detection of Microsatellite Alterations in Plasma DNA of Malignant Mucosal Melanoma Using Whole Genome Amplification

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Abstract: Malignant mucosal melanoma (MMM) still has the poorest prognosis. There is a paucity of molecular biological studies on MMM of the head and neck. We investigated free-circulating DNA microsatellites with loss of heterozygosity (LOH) in the blood of MMM patients. Cancer-related DNA is found in plasma, with cancer patients showing a higher level of free-circulating DNA than normal subjects. However, it is difficult to obtain sufficient amounts of such DNA for PCR analysis. We have searched for ways to improve all stages of such research, and detected new microsatellite alterations by triplicated whole genome amplification (WGA) and triplicated PCR amplification. In order to achieve a better understanding of the extent of the alterations affecting chromosomes we determined the occurrence of LOH at the following gene loci: D1S243, D6S311, D9S161, and D19S246; only 4 out of the 20 microsatellite markers usually used in MMM were used in this study. We determined LOH in 17 MMM patients.

It was possible to confirm LOH on at least one marker in 12 (70.6%) out of the 17 patients. Metastasis or recurrence was confirmed in 3 (17.6%) out of the 17 patients, and all of them were found to have LOH. LOH at microsatellite markers D1S243, D6S311, D9S161 and D19S246 in the plasma of these patients statistically correlated with MMM. The results of this study suggest that these loci are suitable for identifying cancerrelated DNA of MMM, and that analysis of LOH in plasma DNA released into the circulation may be useful as a screening tool.

Key words: Free-circulating DNA, Plasma DNA, Loss of heterozygosity (LOH), Whole genome amplification (WGA), Malignant mucosal melanoma

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