C9. **Flow-cytometry investigation of cutaneous melanoma. Correlations with genomic tests**

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Introduction. Cutaneous melanoma (CM) is below 5% of skin cancer cases, but is responsible for 80% of deaths from these cancers. The prognosis worsens as the lesion extends under the skin due to increased tumor tendency of invasion and metastasis. Evaluation by flow-cytometry of melanoma cells positive for MCSP (Melanoma-associated Chondroitin-Sulfate Proteoglycan), coupled with the identification of mutations in genes associated with cell cycle control and highlighting changes in the products of these genes, may represent indices to assess the evolution of the tumor.

Materials and methods. MCSP+ cells in peripheral blood were identified by flow-cytometry with anti-MCSP–APC antibody (*Miltenyi Biotec*) in 23 MC patients and 5 control subjects. A total of 37 types of mutations in the BRAF, CDKN2A, CTNNB1, GNAQ, HRAS, KIT, KRAS, NRAS, PIK3CA and STK11 genes were analyzed by qPCR-array (Human Melanoma Somatic Mutation qBiomarker - *SABioscience/Qiagen*) on DNA extracted from 8 MC. The products of BRAF, HRAS and KRAS genes were evaluated by ELISA (*MyBioSource*) in the sera of 21 MC patients. Correlations between serum levels of gene products and the presence of circulating MCSP+ tumor cells were performed for 11 patients.

Results. The presence of gene mutations was found in 63% of MC tested cases. The determinations of serum proteins encoded by BRAF, HRAS and KRAS genes revealed increases in 52% patients for BRAF, 10% patients for HRAS and 57% patients for KRAS, representing cases with overexpression of these genes and subsequent increase in blood concentration of gene resulting products. 64% of patients tested for MCSP+ cells showed higher percentages than in controls, indicating the presence of circulating tumor cells. The correlation of this result with BRAF, HRAS and KRAS products concentrations in serum revealed an increase of at least one gene product in 83% of MCSP+ patients, suggesting that mutations in these genes may be associated with metastasis.

Conclusion. The MCSP and ELISA for gene products tests in MC are useful in determining the tumor invasive potential and treatment monitoring in patients with known mutations in genes involved in the development and proliferation of malignant melanocytes.