MELANOMA AND MELANOCYTIC NAEVI

IMPROVED CIRCULATING MELANOMA CELL DETECTION BY A COMBINED MCAM AND ABCB5 ENRICHMENT AND MOLECULAR CHARACTERIZATION OF A PROGNOSTIC BIOMARKER PANEL.

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Melanoma progression involves uncontrolled growth, resistance to apoptosis, invasiveness and angiogenesis, releasing angiogenic factors, disregulating cell-cell adhesion and activating Matrix-Metallo Proteinases, (MMPs). A crucial step in melanoma metastatic processes is the intravascular invasion of neoplastic cells as circulating melanoma cells, CMCs. Specific enrichment and characterization of CMC could be useful to monitor disease status and patient clinical outcome. We documented that MCAM/MUC18/CD146 expression predicts clinical relapse, whereas absence or persistent loss is related to stable disease or to disease-free status. This cell-adhesion-molecule has been shown to be a key oncogene driving melanoma progression and metastasis.

Objective: Assessment of an home-made CMCs enrichment protocol. Final aim of this project is to assess a biomarker expression panel helpful to discriminates high-risk and low-risk patients defined by distinct molecular "CMCs phenotypes".

Materials and Methods: We recruited 13 MM patients (median age 60 yrs) staged ≥pT1b AJCC. CMCs enrichment was performed by a home-made immuno-magnetical selection by using antibody anti-CD146 and anti-ABCB5 (stem-melanocyte epitope) respectively, preceded by CD45 immuno-depletion. After total RNA extraction from rare CMCs we performed a gene expression panel comprising angiogenic factors (VEGF, bFGF), differentiation markers (Tyrosinase, MART1), cell-cell-adhesion-molecules (MCAM/MUC18/CD146 Long, Short and 5'-portion, E-Cadherin, N-Cadherin, VE–Cadherin) and matrix-metallo-proteinases (MMP2 and MMP9).

Results: Preliminary findings documented definition of two distinct CMCS “phenotypes”: “High expressing” CD45-CD146+CMCs and “Low Expressing CD45-ABCB5+CMCs”. Particularly, the CD45-CD146+CMCs showed positive expression for MCAM/MUC18/CD146 5’-portion, Long, and Short isoforms (46%), N-Cadherin VE–Cadherin (39%), VEGF (30%), MMPs (60%) whilst the CD45-ABCB5+CMCs showed low frequency expression for VEGF, MCAM/MUC18/CD146 (7%) and for all the others (not more than 23%). Differentiation markers (Tyrosinase, MART1) were never detected.

Conclusions: Despite the difficulty of isolating rare CMCs, MCAM/MUC18/CD146 as melanoma-specific-antigen results to be correct target for enrichment. Preliminary observation indicates that enriched CD45-CD146+CMCs express a biomarker-panel helpful to characterize melanoma progression.