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Tissue Proteomics of Ovarian Cancer

M. El Ayed(1,3) • D. Bonnel(1) • C. Castellier(1,2) • R. Longuespée(1) • D. Vinatier(2) • A. Kenani(3) • I. Fournier(1) M. Salzet(1) • *(1)MALDI Imaging Team, CNRS, Laboratoire de Neuroimmunologie et Neurochimie Evolutives, FRE CNRS 3249, Université de Lille 1, Villeneuve d'Ascq, France* • *(2)Clinique Gynécologique, Hôpital Jeanne de Flandre, Lille, France* • *(3)Laboratoire de Biochimie , Unité de Recherches "Mécanismes Moléculaires et Pathologies", Faculté de Médecine de Monastir, Tunisie*

Direct analysis of tissues by MALDI MS is an interesting strategy for clinical applications (markers hunting of pathologies) by giving direct information at the tumor level. In fact, in a cancerous tissue, most parts of the tissue do present the same tumoral phenotype. Potential markers can be identified and cross-validated using different tools (IHC, western blots, MALDI imaging) to confirm their presence and localization. We have applied this strategy of tissue proteomics for biomarkers hunting of ovarian cancer 1-3 . Ovarian carcinomas and benign ovaries were directly analyzed by MALDI-TOF-MS. After automatic profiling and mass spectrometry imaging analyses, hierarchical clustering based on principal component analysis in nonsupervised mode was carried out. On the same samples, preparations were performed to investigate peptides, then proteins, followed by high mass proteins, in an automatic profiling to specific signatures for diagnosis. Using tissue bottom-up strategy on tissue digestion, and mass spectrometry imaging after by shotgun sequencing by nalano-LC-IT-MS in MS/MS mode from washing samples from on tissue digested peptides, several biomarkers were found. Comparison of benign and cancerous biopsies according to the different strategies has allowed obtaining a list of potential biomarkers. Among these biomarkers several were identified. One marker is a fragment of immunoproteasome 11S 3, 4. This fragment was found by cross validation to be present in epithelial cells. IHC reveals a change in the addressing of the fragment between cancer and benign samples from nucleus to cytoplasm. However, this fragment was not found in epithelial cells of other cancers such as colon cancers but was also found in uterine cancers demonstrating that PA 28 alpha fragment is specific of genital cancers. This underlines the role of immunity failure (immuno tolerance) in cancer cases with invalidation of the immunoproteasome. Interestingly, using MALDI MSI High mass 5 detection strategy coupled to bottom-up procedures 6, 7, several other biomarkers have been identified. 8 A list of specific biomarkers from the ovarian carcinoma regions was obtained and classified as proteins associated with cell proliferation, involved in immune response modulation, signaling to the cytoskeleton, and tumor progression. These specific biomarkers were then validated by immunocytochemistry using Tag-mass technology, cell biology, and Western blot, and by PCR (using SKOV-3 ovarian epithelial cancer cells) 8. From several candidate proteins, including profilin-1, cofilin-1, vimentin, and cytokeratin 19 involved in intracellular signaling to the cytoskeleton, some are implicated in the conversion of epithelial cells to mesenchymal cell. This clearly demonstrates the interest of the strategy for finding markers 9. Tissue proteomics has higher potential for markers discovery by searching markers directly at the tumor level and ease their tracking in fluids for diagnosis. Such a strategy allows avoiding pitfalls of markers hunting directly from fluids.

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