ImmunoTools *special* Award 2014

Stefania Mitola
Assistant Professor

Experimental Oncology and Immunology, Department of Molecular and Translational Medicine, School of Medicine, University of Brescia, viale Europa 11, 25123 Brescia, Italy

CHARACTERIZATION OF LOW FREQUENCE MUTATION IN CHEMOKINE RECEPTOR FAMILY

Metastasis, the principal cause of death in cancer patients, is the process by which cancer cells leave the primary tumor, travelling across blood and lymphatic vasculature, establishing “beach-heads” in other host organs.

Chemokine Receptors (CKRs) and homeostatic chemokines modulate cancer cell proliferation, survival and senescence and regulate angiogenesis. Besides, CKRs drive cancer cell directional migration and homing; these observations suggest the newly proposed models of “cellular highways”. Indeed, cancer cells overexpressing CKRs are able to follow a gradient of chemokine to their target organ.

Up to now, next generation sequencing (NGS) and gene expression profiling techniques have identified several cases of CKRs mutations, copy number alterations, and deregulated expression in many human cancers. Nevertheless, it is not yet clear if these mutations could imply defects in CKRs signalling in metastatic cells. The difficulties in obtaining and analyzing these data are due to the poor number of cancer samples available and to the low-frequency of the mutations founded.

The current state of the art in analysing NGS data consists in various algorithms for the identification of driver mutations (genomic markers) in genes associated to the cancer. The success of these algorithms depends on the high number of mutations analyzed to produce signatures. Limitations of these procedures occur when, in some cases, it is necessary to identify conserved patterns of low-frequency genomic alterations that the over cited statistical approaches tend to discard: the CKRs mutations represent one of these cases.

To overcame this limit in collaboration with Dr Luca Zammataro (Computational Research Unit, Center of Genomic Science of IIT@SEMM) we developed a trivial form of statistical approach for the analysis of Low Frequency Mutation, (LowMACA) to identify probable

patterns of low frequency mutations that are conserved in CKRs. Our approach takes the information coming from the mutations recorded in different members of CKRs family and collects them on the multiple alignment of the members sequences.

All the mutations selected by our method frequently fall upon specific positions of the consensus impacting four main CKRs functions: ligand binding, G-protein interaction, dimerization, and AP-2 interaction. These mutations may be eventually considered “highly conserved” in cancer. The presence of these mutations can affect the homing of metastatic cells and the recruitment of inflammatory cells into the tumor. These observations lead to the hypothesis that mutations affecting one gene of a family have high probability to be conserved in all the genes of the same family. We can conclude that cancer cells select mutations that produce specific gain of functions, shared by genes within a family, rendering those genes a complex of molecules suitable to become “oncogenes”.

LowMACA has identified six clusters of mutations in common with almost all the CKRs.
In order to characterize the role of the identified mutations in cell motility and invasion we will introduce these in human CKRs cDNA using a quick change lightiming site-directed mutagenesis kit. Then, these CKR mutants will be expressed in endothelial cells or in melanoma B16 cells and assessed for the capacity to modulate chemokines-dependent activity like:

- cell proliferation
- intracellular calcium mobilization
- chemotaxis (Boyden chamber assay)
- receptor internalization
- receptor intracellular signaling
- receptor interaction with intracellular second messengers like AP-2

In order to do these experiments we will need a substantial amount of cytokines making this box valuable. Results from these experiments will contribute to test the reliability of LowMACA tool in human tumor classification and to elucidate the role of low frequency mutation in CKRs in cancer dissemination.