Extracellular cancer signatures as targets for nanotechnology-based diagnostic and therapeutic approaches

The development of metastases is the main cause of cancer death. Indeed, despite recent improvements in both classical chemotherapy and targeted bio-drugs, for many cancer types cure remains anecdotal. The identification of novel targets and/or targeting moieties is therefore an important, still open medical need. The Laboratory of Tumor Microenvironment, where I attended my Ph.D. training, is focused on the identification of proteomic signatures peculiar of pathological sites in oncology, and on the validation and exploitation of peptides as specific addressers for diagnostic and therapeutic compounds.

My project started from the identification, by means of a high-throughput phage display approach, of ~200 peptides that bind specifically to cells of human hepatic metastases secondary to CRC. An *in silico* analysis of this peptide set allowed to define an extracellular protein signature peculiar of the metastasis microenvironment. Among this signature, we described a prototype ligand/receptor pair with a functional role in hepatic homing and colonization (manuscript submitted).

A panel of peptides were further characterized leading to the selection of two metastasis-specific peptides (MTS-p7 and MTS-p8) that were exploited as candidates for a targeted delivery of carriers to the liver metastasis. The main goal of my Ph.D. work was the design and testing of fluorescent core-shell/silica-PEG nanoparticles (SPN) for *in vitro*, *ex vivo* and *in vivo* imaging of CRC micrometastases. In collaboration with the Chemistry Department at the University of Bologna, we prepared SPN containing either Rhodamine B, Cy5Net, or both fluorochromes, which were surface-functionalized with MTS-p7 and MTS-p8 to obtain the nanomodular imaging shuttles MTS-Fluo-SPN. We first tested these objects *ex vivo* on samples of human biopsies by the adaptation of confocal microscopy protocols, demonstrating that the MTS-Fluo-SPN specifically recognize the metastatic tissues, compared to normal samples and to control Fluo-SPN. A further step was to evaluate the applicability of the MTS-Fluo-SPN for *in vivo* imaging, to detect even micrometastases. For this purpose, we set up models of metastatic CRC, by the injection of human cell lines into the spleens of immunocompromised mice. In this system, a primary tumor and several liver metastases develop in less than one month with high reproducibility. MTS-Fluo-SPN injected in the tail vein of mice with hepatic metastases confirmed a specific addressing, with very low background in other healthy and pathological tissues (manuscript in preparation).

One of the described MTS-peptides was further exploited for the production of a Raman spectroscopy-based nanosensor, in collaboration with the Physics Department at the Polytechnic of Torino. MTS-p7 was chemisorbed on Ag nanoparticles synthesized on porous silicon samples with the plasmonic resonance tuned at the excitation energy, and this substrate was used as a bait for a specific anti-MTS-p7 polyclonal antibody. The Raman fingerprint of the biological complex showed that the functionalized nanoparticles have a good selectivity to the target analyte, as required by most of the SERS applications on biological assay. This application will be further developed as a nanosensor to discriminate the fingerprint of different peptides in biological samples (manuscript submitted).

During my Ph.D. training, I was also involved in a collaborative project aimed at the development of peptide-functionalized liposomes for targeted delivery of chemotherapeutics to neuroblastoma, the most common extra-cranial solid tumor of infancy. This project originated from a stage in the Lab of Oncology, Gaslini Children's Hospital, that I attended for the preparation of my Master Thesis. We designed and tested doxorubicin-encapsulating liposomes functionalized with peptide ligands specific for aminopeptidase A (APA), a marker of tumor perivascular cells, and aminopeptidase N (APN), a marker of tumor endothelial cells. We demonstrated that a combined APA/APN targeting is strongly effective in increasing the life span of mice bearing orthotopic models of human neuroblastoma (J Control Release 2010;145:66-73).

In conclusion, during my Ph.D. training I have been involved in a multidisciplinary research team including specialists in medicine, biology, chemistry and physics, with the collective purpose to describe tumor microenvironments and to exploit the deriving molecular findings toward the development of innovative diagnostic and therapeutic tools.