SWISS BRIDGE

INVEST IN CANCER RESEARCH WORLDWIDE

SWISS BRIDGE Award for Cancer Research 2015
Summaries of the two supported research projects

Professor Andreas Trumpp, PhD, Head of the Division Stem Cells and Cancer, Deutsches Krebsforschungszentrum (DKFZ) Heidelberg, receives 250 000 Swiss francs for the project entitled:

Multiomic characterization and targeting of blood circulating metastasis stem cells in breast cancer

Summary

Breast cancer (BrCa) is the predominant type of cancer in women. Although hormone receptor (ER+) positive tumors of the luminal A subtype (low-risk) have a favourable prognosis, luminal B type, HER2+ and triple negative (TN) tumors have a poor clinical outcome. The poor clinical outcome is directly related to their resistance to standard therapy and the development of metastatic disease. Distant metastasis formation is caused by dissemination of tumor cells with clonal capacity (breast cancer stem cells) into the blood circulation followed by extravasation and clonal outgrowth in a distant organ such as lung, liver, bone or brain. We have recently identified blood circulating metastasis-initiating cells (MICs) from ER+ luminal BrCa patients. These MICs have the capacity to re-initiate metastasis in mice, and thus show metastasis stem cell activity. Functionally-defined MICs display an $EPCAM^{+}CD44^{+}CD47^{+}MET^{+}$ phenotype by FACS analysis and their percentage varies between 1%-44% within the circulating tumor cell (CTCs) population, demonstrating a remarkable inter- and intra-patient CTC heterogeneity. Moreover, the presence of $MET^{+}CD47^{+}$ MICs before the onset of metastasis in ER+ luminal patients is associated with a 10.3 year shorter overall survival compared to MET^{neg} $CD47^{neg}$ patients. These data suggest that direct *ex vivo* characterization of blood circulating MICs in BrCa subtypes can have a significant impact on a better understanding of metastasis formation.

To comprehensively characterize CTCs and MICs at the genomic level, we propose in the first aim a thorough multi-level OMICs approach to determine their detailed molecular make-up at various levels of complexity. We plan to generate a resource data set useable by the entire breast cancer community - and beyond - which comprises transcriptomes (RNA-Seq), methylomes (bisulfite sequencing), mutational fingerprints (targeted NGS) and chromatin dynamics (ChIP-Seq) of CTCs and MICs of all major BrCa subtypes isolated by apheresis. Complementarily, we will address the intra- and inter- heterogeneity of patient CTCs by single cell RNA-Seq analysis. We will determine the functional MIC phenotype of TN BrCa and will establish an organoid platform with the aim to culture and expand BrCa cells present in serous effusions (SE) as well as blood-borne CTCs/MICs from patients. Expanded primary samples can then be functionally interrogated (drug sensitivity, clonogenicity and differentiation potential) and associated to the molecular networks employed in them. The organoid expansion protocol will then provide for the first time a way to undertake detailed longitudinal molecular and functional analysis of patient CTCs/MICs before, during and after therapy. Finally, using patient CTCs/MIC-derived organoids in conjunction with the available MIC-mediated xenograft models, any vulnerability newly identified by the multi-omics approaches, as well as already known targets such as MET and CD47 will be explored in interventional-type preclinical trials, with the goal to contribute to the development of better diagnostic tools as well as more effective strategies to target metastatic disease.

The central hypothesis of the project is that we can use primary CTCs/MICs directly isolated from BrCa patients as populations, single cells or organoids to (1) identify central molecular networks critical for metastasis spread and therapy response (2) highlight metastasis pathways

specific for subtypes already known to display different aggressiveness and metastatic proficiency (3) validate CTC-associated molecular alterations in human primary tumor samples and (4) test vulnerabilities in a more advanced pre-clinical setting represented by patient-derived organoids and patient-derived xenografts obtained directly from CTC/MICs.

Professor Joerg Huelsken, PhD, Ècole Polytechnique Fédérale de Lausanne (EPFL), Switzerland, receives 250 000 Swiss francs for the project entitled:

Mechanisms of immune evasion by cancer stem cells

Summary

We had previously identified a sub-population of cancer cells, which have been termed cancer stem cells (CSCs) or tumor initiating cells, as the major driver for tumor progression and metastasis. These CSCs are known to be resistant against a number of classical treatments such as chemo- or irradiation therapy and have been implicated in tumor recurrence. We have now found that these cells also have important immune regulatory functions. Using the 4T1 pre-clinical breast cancer model we observed that the rare population of CSCs is protected from T cell attack whereas all other tumor cells are readily eliminated by CD8+ cytotoxic T lymphocytes (CTLs). Similar results have been reported recently in leukemia. Moreover, we find these CSCs to be responsible for the establishment of an immune suppressive environment. 4T1 tumors show a high degree of immune suppression, however, selective ablation of CSCs triggers activation and expansion of tumor-reactive CTLs. Interestingly, when we genetically engineered T cells to express chimeric antigen receptors (CARs) targeting specifically CSCs, these modified T cells efficiently eliminate cancer stem cells. This clearly demonstrates that mechanisms of immune evasion exist in CSCs, which can only be overcome if T cells are very potently activated as it is the case for CAR-expressing T cells. We now want to better define the immune suppressive activities of CSCs. We will analyse resistance of CSCs to T cell mediated killing in a number of murine and human breast cancer models and we will generate a new spontaneous mouse breast cancer model to study and quantify anti-tumoral immune responses *in vivo*. Using RNAseq we aim to identify mechanisms responsible for this resistance of CSCs against T cell mediated targeting and will develop reagents which can be used to block this CSC capacity for a potential use in cancer therapy.
