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BASTION – FROM BASIC TO TRANSLATIONAL RESEARCH IN ONCOLOGY

Report on the visit of Dr Marzena Łazarczyk at the Center for Molecular Medicine, Karolinska Institutet (Stockholm, Sweden), within the 7PR21/BASTION/WP1 (Twinning)

From 30 January till 30 July 2015 I stayed at Cell and Molecular Immunology Department at Center for Molecular Medicine (CMM) in Stockholm headed by Cecilia Söderberg-Nauclér.

- ***Background of research interest***

Colorectal cancer is the third most frequently diagnosed cancer in men and the second in women. According to WHO GLOBOCAN database the highest incidence rates are in Australia, New Zealand, Europe and North America and the lowest - in Africa and South-Central Asia. Application of available medicines or physical methods for colon cancer treatment in routine clinical practice is associated with serious side effects experienced by patients, therefore the risk-benefit assessment is still burdened with their high toxicity/harmfulness. Available scientific reports emphasize the role of chemokines and chemokine receptors signaling in cancer development. The purpose of my studies was to identify the role of chemokine 9 (CCL9; alternative name: macrophage inflammatory protein 1γ; MIP-1γ)in colon cancer using mouse models. Although there is no dataavailable in this field, our preliminary results inmouse colon cancer CT26 cells and MSCs co-cultures indicated that relationship between CCL9 and colon cancer may exist.

- ***Research tasks***
  - Generation of CCL9-overexpressing CT26 cell line using*Ccl9* gene enhancement technology (performed during first twinning stay at CMM).
  - *In vitro* studies inunmodified CT26 cells exposed to recombinant mouseMIP-1γ protein or supernatants collected from CCL9-enhanced CT26 cell culture using proliferation, invasion and migration tests (started and continued within two twinning stays). Concomitant *in vitro* studies utilizing CCL9 receptor (CCR1)inhibitor -BX471.
  - *In vivo* studies in mouse models (performed).

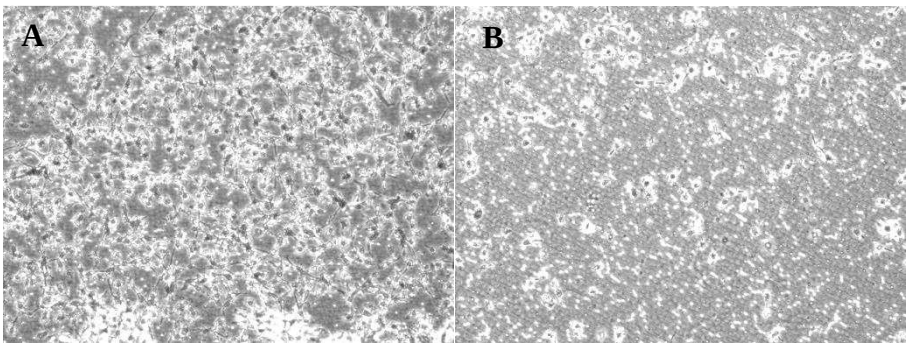
- ***Incorporated research methods***
  - ELISA for CCL9 detection in supernatants collected from control and CCL9-enhanced CT26 cell cultures;
  - MTT proliferation assay usingCell Titer 96 AQueous One Solution Reagent;
  - scratch assay for migration study according to Chun-Chi Liang et al., Nature Protocols 2, - 329 - 333 (2007); images performed usingOlympus CKX41 microscope and Image-Pro Plus 7.0 software and analyzed with ImageJ software;
  - QCM 24-well Colorimetric Cell Migration Assay application for migration analysis;
  - invasion assay using matrigel-loaded transwell membranes, DAPI and confocal microscope (Zeiss) for imaging;
  - RNA isolation using QiagenRNAeasy kit for PCR (transcripts for chemokine receptors detection);
  - microarray analysis (to be performed on collected tumour tissues from *in vivo* experiments and on generated CCL9-enhanced CT26 cell line)
  - *in vivo* study on BALB/c mice injected subcutaneously with CCL9-enhanced CT26 or blank control CT26 cells (repeated 2 times with different number of cells inoculated) for tumour excision and fixation / organs collection for metastasis detection (done).

A B

Confocal microscope at CMM (A)

Cell culture room at CMM (B)

- ***Selected in vitro results***



Transwell membrane with migrated blank control cells (A) and CCL9-enh. CT26 cells (B)

- ***Selected in vivo results***

In vivo studies in mice with subcutaneous injection of modified and blank control CT26 cells revealed significant differences in tumour growth between experimental groups: animals receiving control cells developed much larger tumours than mice injected with CCL9-enhanced CT26 cells as measured by caliper every 2 days. In addition, tumours appeared two times more frequently in control group than in animals inoculated with CCL9-enhanced CT26 cells. Tumour tissues and organs with suspected metastasis were fixed in formalin and frozen for inflammatory cells and lymphatic vessel immunodetectionas well as microarray analysis.

- ***Cooperation with team members and future perspectives***

Apart from studies on CCL9 chemokine in cancer, I supported team members of Piotr Religa's group in their research performing RNA isolations,immunohistochemical staining of several markers: CD45, CD68, LYVE1 on paraffin sections, doing experiments on CMV virus involvement in colon cancer employing mouse models with antiviral treatments and pilot study on BALB/c nude mice injected subcutaneously with human breast cancer MCF-7. Mutual cooperation with CMM members will be continued in the nearest future as well as project on CCL9 in Warsaw.

The time I spent in Swedish science entity, being one of the world's leading medical research centers was very fruitful and gave to me awesome opportunity to broaden my knowledge and get familiar with new scientific tools/methods.