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BASTION - FROM BASIC TO TRANSLATIONAL RESEARCH IN ONCOLOGY

Report from active participation in Keystone Symposia Conference "Inflammation, Infection and Cancer (X1)", 9 - 14 March 2014, Whistler, Canada – Tomasz Rygiel

Keystone Symposia on Molecular and Cellular Biology is a nonprofit organization with a 42-year history of convening open, peer-reviewed conferences that connect the scientific community and accelerate life science discovery.

This year, Inflammation, Infection and Cancer (X1) was held jointly with Immune Evolution in Cancer (X2) in Whistler on March 9-14, 2014. The major topics involved influence of immune cells on tumor development, modulation of immune responses in anticancer therapies and importance of inflammation process in tumor development. As always majority of talks were given by invited top class experts in the field.

During the poster session (3) held on March 12th, I had presented our results at the poster entitled: "CD200-CD200R pathway influences tumor growth by infiltrating myeloid cells", Authors: Zofia Pilch, Katarzyna Roszczenko, Linde Meyaard, Jakub Gołąb, Tomasz P. Rygiel. Our major finding was that modulation of CD200-CD200R signaling affects infiltration of tumors by immune cells and the speed of tumor growth in experimental mouse tumor models.

Due to the policy of organizers (Keystone Symposia), I was unable to take a picture during the poster session. No video equipment, cameras or any type of recording device is allowed in the meeting rooms or poser sessions. Instead, I supply the page 100, from the conference book that contains abstract of my poster.

Poster Session 3: Wednesday, March 12

X1 3013 Novel inhibitor of Programmed Cell Death (PD-1) Expression in the Cancer immunotherapy

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Despite its central importance of programmed death 1 (PD-1) expression on CD8 T-cells in cancer immunotherapy, the proximal signalling pathway that couples the T-cell receptor to PD-1 expression has yet to be defined. In this study, we have identified a novel upstream mediator that acts as the central upstream signaling node in the control of PD-1 transcription. Its inhibition augmented transcription of the transcription factor Tbet that otherwise suppressed the transcription and expression of PD-1 (i.e. suppresses a suppressor). This was observed using competitive inhibitors, or its down-regulation by siRNA that markedly inhibited PD-1 transcription leading to the enhanced *in vivo* clearance of subcutaneous growth and pulmonary metastases of B16 melanoma B16 mouse melanoma cells and in mouse lymphoma EL4 tumor-bearing mouse models. The effects involved primarily enhanced ability of CD8+ T-cells to kill tumour targets. Our findings have identified the central upstream regulator of PD-1 transcription and have demonstrated the *in vivo* applicability of a novel drug-based approach for enhanced *in vivo* CTL responses to cancer.

X1 3014 CD200-CD200R pathway influences tumor growth by infiltrating myeloid cells

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Tumor microenvironment is to a great extend shaped by infiltrating immune cells. Frequently it is characterized by immunosuppressive inflammation that promotes angiogenesis and tumor metastasis. Previously, we showed that lack of CD200R signaling increases resistance to development of chemically induced endogenous skin tumors. However, the mechanism responsible for that effect is still elusive. Newer less, disruption of CD200-CD200R signaling is currently tested therapy for B cell malignancies.

In this project we hypothesized that inhibition of CD200-CD200R signaling may in some conditions, stimulate rather than inhibit tumor growth. We tested multiple mouse syngeneic tumor models in the context of CD200R inhibition or over-stimulation.

We noticed increased tumor growth of B16F10 melanoma in CD200-defficient mice, measured by bioluminescence and tumor foci count in the lungs. Whereas adverse treatment, triggering of CD200R with an agonistic antibody, did not affect growth of B16F10 tumors. Further, we showed that CD200R affects infiltration of the tumor-bearing lungs by $CD11b^{+}Ly6C^{+}$ myeloid cells. CD200R-stimulation inhibits, while CD200R-inhibition increases infiltration of $CD11b^{+}Ly6C^{+}$ cells to the tumor-bearing lungs in contrast to tumor free spleens of B16F10 melanoma bearing mice. These results suggest that disruption of CD200-CD200R signaling affects tumor growth, possibly by regulation of tumor targeting myeloid cells.

This research is founded by (NCN 2011/03/D/NZ6/03685, FNP HOMING PLUS/2011-4/10)

X1 3015 LYVE-1, a tumor- associated macrophages marker in mouse and human and its role in cancer biology

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Tumor-associated macrophages represent macrophages involved in tumor initiation, tumor progression and metastasis. We have identified the lymphatic endothelial cell marker Lyve-1 to be expressed by tumor-associated macrophages.
In this study we found Lyve-1 to be induced in human peripheral blood monocytes (pBMs) *in vitro* by a combined stimulation with M-CSF, dexamethasone and IL-4.
In addition, Lyve-1 expression in pBMs was also achieved by co-culturing human pBMs with WM115 melanoma cells. WM115 cells were originally derived from a primary melanoma, while the WM266.4 cell line originated from a metastasis of the same patient. Co-cultivation of WM266.4 cells with pBMs did however not induce Lyve-1 expression *in vitro*. To verify the *in vitro* findings in an *in vivo* setting, different pathological stages of melanoma were immunohistochemically stained with Lyve-1 antibody. Lyve-1 was thereby expressed by a subpopulation of TAMs in all tumor stages. In order to assess, whether Lyve-1 has a tumor promoting function, the lewis lung carcinoma cell line LLC, which has been shown to be strongly infiltrated with Lyve-1 positive macrophages, was injected in the right flank of Lyve-1 knockout and control mice. After 14 days of tumor growth, the tumors showed no difference in final tumor end weight. Immuohistochemical analysis however revealed less caspase3 positive necrotic areas in LLC carcinomas of Lyve-1 knockout mice. This finding did not correlate with a difference in vessel or macrophage density.

These results suggest that the presence of Lyve-1 might enhance the susceptibility of tumor cells for apoptotic or necrotic stimuli. Further studies are needed to better understand this effect of Lyve-1 for the tumor biology.

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X1 3016 Searching for the Key Factors for Adoptive T Cell Therapy for a B Cell Lymphoma

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Adoptive T cell therapy (ACT) is the infusion of cytotoxic T lymphocytes (CTL) specific for tumour-associated antigens into tumour-bearing hosts. While this approach has shown promise in patients with advanced disease, its effectiveness remains limited due to poor survival of the transferred T cells.

To characterise parameters that influence ACT we utilized a mouse B-cell lymphoma (derived from Eu-myc transgenic mouse) expressing ovalbumin (OVA) as model tumor-antigen. To mimic ACT OVA-specific cytotoxic CD8+ T lymphocytes (OT-I CTL) can be generated *in vitro* and inoculated into Eu-myc-OVA tumour-bearing mice. Previously, we demonstrated that OT-I CTL become inactivated following transfer into mice bearing a large tumour mass. This inactivation was antigen-specific and mediated by direct antigen recognition on the tumour cells.

Here, we aimed to investigate the role of different factors that affect inactivation of adoptively transferred anti-tumour specific CTL. We have sorted T cells from tumour bearing mice and did RNA sequencing to identify genes that could be key in their impaired function. Our data demonstrate that there are clear pathways down regulated in impaired T cells like interferon gamma (IFN-γ) related genes and other up regulated related to survival of the T cells... Overall these results can help us understand how to design the best strategies to achieve effective cancer immunotherapies.