ImmunoTools *special* Award 2017

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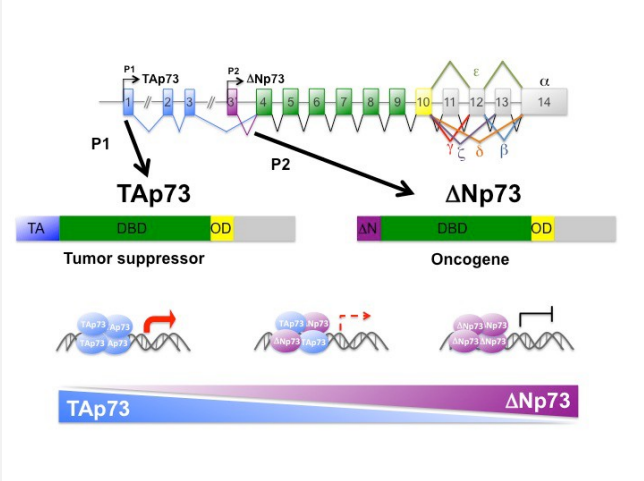
Investigating the role of p73 isoforms in controlling tumor microenvironment

Tumor development is not only dependent on cell intrinsic activation of proliferation and inhibition of senescence and/or apoptosis. It is becoming increasingly clear that the interactions between tumor cells and the surrounding stroma play a critical role for maintenance, growth, and spread of the tumor. Tumor cells release factors that not only induce tumor angiogenesis but also attracts infiltrating leukocytes to the pro-inflammatory tumor microenvironment. Many immune cells have anti-tumorigenic functions but are re-educated by the tumor cells to induce blood vessel formation and promote metastasis thus supporting tumor progression. The p73 gene encodes for two isoform families, TAp73 isoforms that act as tumor suppressors, and the ΔNp73 isoforms that have oncogenic properties. In this project we propose to investigate how tumor cells expressing different p73 isoforms control the tumor microenvironment, specifically, we will study the effect on macrophage infiltration and polarization, as well as, NK cell function. Increasing our understanding of the molecular mechanisms controlling tumor development will ultimately lead to better tools for diagnosis as well as the ability to develop novel, more specific, cancer drugs in the future.

The p73 gene is a member of the p53 gene family that include the p53, p63 and p73 genes encoding proteins that share structural and functional homology, and act as transcription factors to regulate cellular proliferation, differentiation and death. In addition to full-length proteins that act as transcription factors (p53, TAp63 and TAp73) the p53, p63, and p73 genes also encode several different N-terminally truncated isoforms due to



usage of an internal promoter (ΔN). The ΔN isoforms block the transactivation activity of the full-length proteins in a dominant-negative fashion thus acting like oncogenes. Furthermore, alternative splicing of C-terminal exons adds additional isoforms (α, β, γ, δ etc). In contrast to p53, the p73 gene is rarely found mutated in tumors, instead it has been suggested that it is a shift in the balance from expression of TAp73 isoforms to ΔNp73 isoforms that is the tumorigenic event. TAp73 has been found silenced through methylation in acute lymphoblastic leukemia, Burkitt's lymphoma, and NK cell lymphomas. In addition, elevated level of ΔNp73 has been found in breast, colon, lung, liver, ovarian and cervical cancers, as well as, malignant melanoma, neuroblastoma and medulloblastoma, and is correlated with chemotherapeutic failure and poor patient survival.

To study the impact of the different p73 isoforms on immune cell infiltration and polarization in the tumor microenvironment we will establish an orthotopic murine breast cancer model. In short, we will inject TAp73$^{+/+}$ or $^{-/-}$ murine breast cancer cells into the fat pad of immunocompetent mice. The tumors will be harvested and immune cells will be stained to perform flow cytometry. For macrophage infiltration we will select for live/CD45^{+} cells and gate for CD11b^{+}Gr1^{-}F4/80^{+} cells. To further investigate polarization of these cells we will use markers such as CD80, CD86, MHC II, CD204, CD206, Ly6c.

Additionally, we want to research if there is a difference in cytokine and chemokine release of TAp73$^{+/+}$ or $^{-/-}$ tumor cells. Therefore, we plan to perform ELISA assays for various relevant cytokines/chemokines including IL-6, TNF-a and MCP1/CCL2. Regarding NK cell infiltration and activation we will take tumors and use flow cytometry to sort for NK cells in the microenvironment using a NK cell pan marker. We will then look specifically at activating and inhibitory NK cell ligands. Furthermore, we want to co-culture TAp73$^{+/+}$ or $^{-/-}$ tumor cells with IL-2 or IL-15 activated NK cells to investigate cytotoxic potential.

Overall, we expect that this project leads to advanced understanding how p73 isoforms influence immune cells in the tumor microenvironment and that this knowledge in the future will help improve drug design.

ImmunoTools *special* AWARD for **Johanna Wolfsberger**

includes 25 reagents

FITC - conjugated anti-mouse CD4, CD45, Gr-1, Annexin

PE - conjugated anti-mouse CD8a, CD80, NK-cells

APC - conjugated anti-mouse CD11b, NK-cells

mouse ELISA-set (for one 96 plate): mouse IL-6, mouse TNF-a, mouse GM-CSF

recombinant mouse cytokines: rm IL-2, rm IL-15, rm MCP-1 (CCL2), rm M-CSF

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