Abstract of dissertation

Resistance against chemotherapy is a central problem in the current treatment of B cell malignancies. Only in the last two decades the importance of the tumor microenvironment for progression and therapy outcome has been revealed. But still up until now, only little is known about the exact mechanisms. Varying between tumors, the microenvironment consists of many different cell types which can either support malignancy and enable relapses after treatment or be used to induce an anti-tumor response. Macrophages are highly present in the microenvironment of most tumors – designated as tumor associated macrophages (TAM) – and are often correlated to a poor prognosis.

We could show that macrophages are an essential element in the synergistic response to the administration of the DNA damaging agent cyclophosphamide and the monoclonal antibody alemtuzumab which strongly increased tumor clearance and prolonged overall survival in the refractory humanized BCL2-MYC double hit lymphoma hMB mouse model.

The alkylating agent cyclophosphamide is inducing the secretion of distinct cytokines containing VEGF-A, CCL4, IL8, IL10 and TNFα by the leukemic cells which in turn leads to a repolarization of tumor associated macrophages from a suppressive to an activated phenotypic state. This is designated as acute secretory activating phenotype (ASAP).

In this work, we show for the first time the importance of the DNA damage pathway for the generation of the ASAP. We display that deregulation in form of downregulation of key player like ATM, DNA-PK or p53 in the leukemic cells prevent the formation of the stimulatory ASAP effect on macrophages phagocytosis capacity.

Here, p53 seems to possess a central regulating role. In *in vitro* systems of the hMB cells as well as using human CLL patient cells, cells with downregulated/mutated p53 prevent the induction of the stimulatory, cytokine induced effect on macrophage phagocytosis in response to combination treatment as seen with their respective control conditions. This is underlined by observations made by treating TCL1/p53$^{wt/wt}$ as well as TCL1p53$^{-/-}$ mice. In line with the hMB system and CLL patient samples, cyclophosphamide treated TCL1p53$^{-/-}$ mice failed to induce an antibody mediated stimulatory effect on macrophage phagocytosis capacity as seen with TCL1/p53$^{wt/wt}$ mice.

These observations are supported by clinical studies describing a strong negative impact of TP53^{mut} in CLL patients treated with FC or FCR affecting progression free- and overall survival and indicates the potential clinical relevance of this work.

Furthermore, we could demonstrate that formation of the ASAP is mechanistically restricted to antibody mediated phagocytosis and is not only based on the macrophage reprogramming by leukemic cell induced cytokine secretion, but also by direct effects of cyclophosphamide on the macrophages. More precisely, we see that the administration of the low dose cyclophosphamide in vitro equivalent agent mafosfamide is increasing the phagocytic capacity *in vitro* and acting on the macrophages by changing their size and appearance.

Taken together we display the importance of p53 for the generation of the ASAP in different models *in vitro* and *in vivo* which is a process much more complex than described until now by taking both sides of interaction into account the leukemic, as well as the macrophage part.

Our ongoing research will help to elucidate the process by which p53 regulates the ASAP response and offers the possibility to reveal new treatment approaches for refractory patients.