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Background: Systemic mastocytosis (SM) is characterized by clonal mast cell (MC) infiltration in the bone marrow (BM). Most patients harbor the activating *KIT* mutation D816 V. Cytokines are substantially involved in the interactions between neoplastic cells and the BM microenvironment in SM and in classical myeloproliferative neoplasms (MPN) and have been recognized as a major trigger of disease evolution. The pro-apoptotic/anti-proliferative and pro-inflammatory cytokine tumor necrosis factor alpha (TNFa) has been identified as an important driver for the expansion of *JAK2* V617F+ clones and the establishment of MPN.
Aims: Investigation of the potential role of TNFa in disease evolution of SM patients.
Methods: In vitro and in vivo experiments with MC lines and primary material of SM patients.
Results: In a first step, lentivirally-driven expression of the KIT D816 V mutant gene in the human Mo7e cells suggested an oncogene-dependent regulation of the production of TNFa. Correspondingly, the human neoplastic *KIT* D816V+ MC lines HMC-1.2 and ROSA$^{KIT\ D816V}$ expressed and secreted substantial amounts of TNFa and inhibition of KIT D816 V either by the recently approved multi-kinase inhibitor midostaurin or by RNAi-mediated knockdown of *KIT* significantly reduced expression of this cytokine. Mechanistically, we studied auto-/paracrine effects of TNFa on the clonal selection of neoplastic MC using *KIT* D816 V negative (ROSA$^{KIT\ WT}$) and positive (ROSA$^{KIT\ D816V}$) subclones of ROSA cells. TNFa reduced proliferation of ROSA$^{KIT\ WT}$ cells (84.9% ± 4.6% of control, p<0.01) whereas ROSA$^{KIT\ D816V}$ cells were resistant to TNFa (102.7% ± 3.7% of control, n.s.). Preliminary data also indicate a relative resistance of primary neoplastic cells obtained from *KIT* D816V+ SM patients against TNFa in colony-forming unit (CFU) assay. To determine the mechanism of TNFa resistance in KIT D816V+ cells, we screened for key regulators of apoptosis and identified the anti-apoptotic protein survivin to be highly upregulated in *KIT* D816V+ ROSA cells upon TNFa treatment but not in *KIT* D816V- ROSA cells. We hypothesize that *KIT* D816V+ MC produce substantial amounts of TNFa that induces apoptosis and suppresses proliferation of normal BM cells while MC themselves are relatively resistant via upregulation of survivin. In a next step, we studied the effect of TNFa on growth of neoplastic MC *in vivo* in a xenotransplant model using ROSA$^{KIT\ D816V}$ cells with and without knockout of TNFa in NOD-SCID IL-2Rg-null (NSG) mice. CRISPR/Cas9-mediated knockout completely abolished expression and secretion of TNFa in ROSA$^{KIT\ D816V}$ cells and resulted in a significantly longer survival of mice compared to mice injected with TNFa expressing controls (median 49 vs. 58 days, p < 0.05). Finally, the clinical relevance of TNFa expression was studied in a SM patient's cohort (n = 45). TNFa levels in sera of patients were significantly higher (median 1.95, range 0.72–16.64 pg/ml, p = 0.002) compared to age- and sex-matched controls (median 1.49, range 0.51–5.63 pg/ml). Importantly, when we stratified SM patients into those with clearly elevated TNFa levels and those with TNFa levels within the reference range of controls (≤3.63 pg/ml) a significantly lower overall survival was observed for SM patients with high TNFa serum levels (median 6.3 years vs. not reached, p < 0.005).
Summary/Conclusion: In summary, we have identified TNFa as a critical, KIT D816V-dependent, cytokine-mediator that promotes clonal expansion of *KIT* D816V+ MC and may thereby be involved in clonal evolution and disease progression in SM.

S889 GRANULOCYTES AND MONO-NUCLEAR CELLS FROM PATIENTS WITH MYELOPROLIFERATIVE DISORDERS HARBOUR PERSISTENT EVIDENCE OF DNA DAMAGE AND TP53 PATHWAY ACTIVATION THAT CORRELATES WITH JAK-STAT ACTIVATION

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Background: The BCR-ABL-negative myeloproliferative neoplasms (MPN) are characterised by constitutive activation of the JAK-STAT pathway in haematopoietic progenitor cells (HPC), typically resulting from somatic mutations in one of three genes; JAK2, MPL or CALR. It is known from murine models that activation of the JAK-STAT pathways results in hyperproliferation of HPCs in the bone marrow niche, and it has recently been shown that this leads to exhaustion and premature aging in a percentage of HPCs. The impact of the activation of TP53 in the context of cells harbouring MPN-driver mutations remains unclear however, and whether these 'TP53-high HPCs' contribute to the mature cell populations in MPNs or are out-competed by 'TP53-low', HPC remains unknown.
Aims: To determine the effect of JAK-STAT activation on DNA damage and TP53 pathways in mature myeloid cells of patients with myeloproliferative disorders.
Methods: Peripheral blood samples from patients with essential thrombocythaemia (ET, n = 10), polycythaemia vera (PV, N = 11) and myelofibrosis (MF, n = 7) as well as healthy controls (NC, n = 11) were fractioned by gradient separation and bead selection into granulocyte (PMN), mononuclear (MNC) and T-cell (TC) fraction. Transcriptomic profiling was performed by expression array and validated by RNA-seq in a subset of patients. Cell fractionation and purity was confirmed by computational decomposition and supervised differential expression, co-expression and gene set enrichment analysis was then performed to identify disease specific pathway mis-regulation.
Results: Both PMN and MNC fractions displayed strong enrichment for hallmark TP53 pathway activation and DNA damage pathways. As expected, no TP53 pathway activation was seen in the T-cell fraction, confirming that this is restricted to the myeloid compartment. Interestingly, TP53 pathway activation directly correlated with JAK-STAT pathway activation, suggesting the p53 activation results directly from the effect of the MPN-driver mutations.
Given that TP53 is capable of activating a number of biological processes including cell cycle arrest, apoptosis and autophagy we looked at expression levels of a number of transcriptional targets of TP53. There was significantly increased expression of the cyclin-dependent kinase inhibitor p21 (CDKN1A) a well-known transcriptional target of p53, in the MNC fraction in all disease groups, and in the PMN fractions of PV and MF. Additionally, we noted upregulation of anti-apoptotic BCL-XL (BCL2L1) in a manner that mimicked CDKN1A upregulation.
Summary/Conclusion: These data show that mature myeloid lineages in patients with MPNs harbour activation of DNA damage and TP53 pathways with transcriptional activation of down-stream effectors in PMN and MNC compartments that correlate with JAK-STAT activation. Typically, engagement of TP53 pathways serves to limit proliferation of mutation harbouring cells, however the upregulation of anti-apoptotic genes may offer a potential mechanism for the persistent of these cells. These data suggest that MPN clones may develop mechanisms that permit escape from TP53 mediated death, and offers potential insight into novel therapeutic targets.

Stem Cell Transplantation - Experimental

S890 NEW WHOLE GENOME ASSOCIATION SCANNING APPROACH FOR THE DISCOVERY OF HLA CLASS I-RESTRICTED MINOR HISTOCOMPATIBILITY ANTIGENS

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