Karolinska Institutet

INSTITUTIONEN FÖR ONKOLOGI-PATOLOGI

Studies on the mechanisms of sorafenib-induced cell death

AKADEMISK AVHANDLING
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Abstract

In 2008, 26% of all deaths were cancer-related, making this group of diseases the second leading cause of death in the EU countries. Derailment of tyrosine kinase signaling is one important pre-requisite towards tumorigenesis. Small molecular inhibitors of receptor tyrosine kinases (RTKs) are a new type of targeted therapy and they are increasingly used as a core component of personalized cancer therapy. The main aim of this thesis is to investigate the anti-cancer effects of the multi tyrosine kinase inhibitor (TKI) sorafenib in hematological and solid tumors.

In the first study, we found that sorafenib is particularly effective in inducing cell death in a panel of human myeloma cell lines. We investigated the mode of cell death induced by sorafenib and found that this TKI induces both caspase dependent and caspase independent cell death. Furthermore, sorafenib induces autophagy in some human myeloma cell lines, myeloma patient samples and mouse myeloma cells and co-treatment of myeloma cells with sorafenib and autophagy inhibitors potentiates the cytotoxic efficacy of sorafenib. Importantly, sorafenib induced cell death in freshly isolated $CD138^+$ multiple myeloma cells from newly diagnosed patients chemotherapy naïve as well as bortezomib resistant patient samples. We investigated the efficacy of sorafenib in the 5T33MM mouse myeloma model and found that this TKI lead to significantly increased survival, reduced tumor growth and decreased serum M component.

In the pertaining studies we investigated the efficacy of sorafenib against prostate cancer cell lines. In the second study we demonstrated that sorafenib caused a dose-dependent decrease in cell viability in two hormone refractory and one hormone responsive prostate cancer cell lines.

In the third study we further investigated the signaling cascades inhibited by sorafenib leading to cell death in prostate cancer cell lines (22Rv1 and PC3). Activation of caspases and downregulation of Mcl-1 are seen in both cell lines. However we found that distinct upstream signaling cascades are activated in these two prostate cancer cell lines which are differentially affected upon treatment with sorafenib. In 22Rv1, ERK1/2 is constitutively phosphorylated and active whereas in PC3 cells it is not active. In contrast, Src and AKT were constitutively active in PC3 cells but not in 22Rv1 and treatment with sorafenib could inhibit these kinases in PC3 cells. In both cell lines, sorafenib induces autophagy and inhibition of autophagy potentiates the cytotoxic efficacy of sorafenib. PC3 and 22Rv1 cells could further be rescued from sorafenib-induced cell death when co-cultured with cancer associated fibroblasts. This protection could be overcome by co-treatment with ABT737 (a Bcl-2/Bcl-xL inhibitor), suggesting that these anti-apoptotic proteins are, at least in part, responsible for the rescuing phenotype observed upon co-culture with cancer associated fibroblasts.

In a fourth study we found that even though DU145 cells do not express *ATG5* they undergo autophagy upon treatment with sorafenib or bafilomycin A1. Interestingly, we showed that sorafenib-induced autophagy in DU145 cells is cytotoxic and the cell death observed could be inhibited by the exogenous re-constitution of Atg5 expression. We found that treatment with molecular or chemical inhibitors of RIPK1 suppressed the observed cell death. Collectively our data suggest that in Atg5-deficient cells autophagy is cytotoxic and the ensuing cell death is executed by the necroptotic program.

In summary, these data identify some molecular mechanisms and requirements for the successful usage of sorafenib as a putative anti-cancer treatment against multiple myeloma and prostate cancer.