Sunitinib inhibits AXL phosphorylation in tumor cells

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Background Sunitinib is a multi-targeted tyrosine kinase inhibitor (TKI), that is used for treatment of patients with advanced renal cell cancer (RCC). Drug resistance to sunitinib is a major clinical problem. We recently reported that continuous exposure to sunitinib causes acquired resistance of tumor cells. The receptor tyrosine kinase Axl has been implicated in acquired resistance to multiple TKIs, including erlotinib and lapatinib. In addition, sunitinib was previously reported to be a potential inhibitor of Axl catalytic activity. We therefore investigated the potential role of Axl in sunitinib resistance.

Methods 786-O cells resistant to sunitinib were obtained by continuous exposure to the drug as previously published (Gotink et al. Clin Cancer Res 2011). AXL expression was studied in 786-O cell lines and EC-RF24 endothelial cells by RT-PCR and Western blot. Sunitinib and AXL inhibitor R428 sensitivity of cells transfected with siRNAs against AXL or scrambled siRNAs was determined by MTT cell viability assays. For kinase activity experiments recombinant AXL and tyrosine peptide arrays (PamGene, ‘s Hertogenbosch, the Netherlands) were used. Phospho-AXL and total AXL in cell lysates were measured by ELISA. Results Axl phosphorylation in vitro in both 786-O and EC-RF24 cells was dose-dependently inhibited by sunitinib, with a 50% inhibitory concentration of 5 µM. Activity of recombinant Axl kinase was measured on tyrosine containing peptide arrays in presence of 0, 1 or 4 µM sunitinib or 2 µM R428. Sunitinib induced dose-dependent inhibition of Axl activity, where 2 µM R428 blocked all peptide phosphorylations. In addition, 786-O sunitinib resistant cells (786-O SUN) showed a 4-fold overexpression of Axl protein compared to 786-O parental cells. However, gene silencing of AXL in 786-O SUN cells by RNA interference did not sensitize the cells to sunitinib. Sunitinib resistant cells were shown to be cross-resistant to the Axl inhibitor R428 with respective IC50 values of 0.9 µM (±0.1 µM) and 1.8 µM (±0.1 µM). Conclusion Phosphorylation of Axl is inhibited by sunitinib in both 786-O RCC and EC-RF24 endothelial cells. Despite its overexpression in sunitinib resistant cells, gene silencing of AXL did not sensitize 786-O resistant cells to sunitinib, indicating that sunitinib resistance is not mediated by AXL.