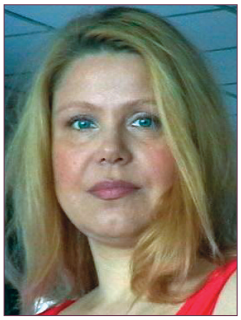
Axl as a Therapeutic Target in Merlin-Deficient Tumours



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Deficiency of a tumour suppressor Merlin leads to the development of tumours of the nervous system such as schwannomas, ependymomas and meningiomas occurring spontaneously or as a part of a hereditary disease neurofibromatosis type 2 (NF2) [1,2]. Current therapies surgery and radiosurgery are only partly effective and new treatments are urgently needed for this group of tumours. Merlin loss is also found in a proportion of other cancers e.g. mesothelioma, melanoma, breast cancer and glioblastoma. Our group successfully studies pathobiology of tumours caused by Merlin mutations [3-10] and aims to find molecules involved in tumour development which could be targeted by specific pharmacological inhibitors. Using our human in vitro model for Merlin-deficient tumours, comprising human primary schwannoma cells, we found that Merlin deficiency results in strong overexpression and activation of platelet-derived growth factor receptors (PDGFR) [3], insulin-like growth factor I receptor (IGF-IR) [6], Integrins [11] and ErbB2/3 [4, 12] leading to strong activation of the downstream signalling pathways such as ERK1/2, AKT1/2, JNK, FAK/Src, Wnt and increased proliferation, cell-matrix adhesion and survival in schwannoma [3-5, 10]. Importantly drugs, such as Sorafenib, Nilotinib, Imatinib, Lapatinib, BEZ-NVP235, R1507 were then tested in our human in vitro model and some of the most promising taken further in to clinical trials [3, 13]. Despite successful studies and detection of good therapeutic targets to treat schwannoma and other Merlin-deficient tumours a comprehensive dissection of signalling matrix involved in tumour development is needed. Inhibition of a single pathway may create a feedback loop towards activation of alternative pathways contributing to tumour development. We have therefore investigated additional mechanisms contributing to schwannoma development. Tyro3 (Sky), Axl and Mer are members of the TAM family of receptor tyrosine kinases shown to be overexpressed in cancers, being markers for poor prognosis and correlating with multi drug resistance (MDR).They also contribute to tumourigenesis by regulating migration and invasion, angiogenesis, cell survival and tumour growth. TAM family receptors are significantly overexpressed in schwannoma tissues [4]. The relevance of Axl in merlin-deficient tumours is underlined by findings showing that Axl

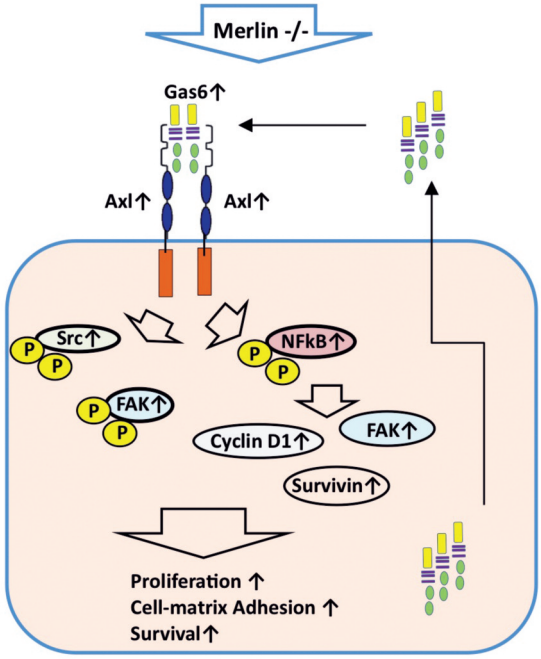

Figure. Merlin deficiency (Merlin -/-) causes increased expression and activation of Axl followed by strong phosphorylation/activation of Src, FAK and NFkB, increased expression of cyclinD1 and survivin and potentiated proliferation, cell-matrix adhesion and survival of schwannoma tumour cells. Gas6 is released and acts in auto/paracrine manner.

is negatively regulated by Merlin and positively regulated by E3 ubiquitin ligase CRL4DCAF1. Merlin seems to inhibit E3 ubiquitin ligase CRL4DCAF1, which is responsible for tyrosine kinase receptors expression changes in Merlin-deficient tumours [14]. The ability of Axl to positively regulate oncogene Yes-associated protein, a downstream member of Hippo pathway known to be under Merlin regulation in schwannoma and involved in increased proliferation of meningioma and mesothelioma, further support for a potential role of Axl in Merlin-deficient tumours [15]. Moreover, TAM family receptors' agonist Gas6 stimulates human Schwann cell proliferation in vitro via Axl and Tyro3 [16]. Using our human schwannoma in vitro model, we demonstrate strong overexpression of all three members of Axl, and the ligand Gas6 in human schwannoma. We show that Gas6 is mitogenic and increases schwannoma cell-matrix adhesion and survival acting via Axl in schwannoma cells. Furthermore, Gas6 signalling via specifically Axl involves focal adhesion kinase (FAK) and Src, but not the ERK1/2, JNK1/2 and AKT signalling

pathways. We also demonstrate the role of NFkB, which regulates Gas6/Axl mediated overexpression of survivin, cyclin D1 and FAK leading to enhanced survival, cell-matrix adhesion and proliferation of schwannoma. NFkB expression was found to be Merlin dependent and its activity depended on Axl. We thus suggest Axl as a promising therapeutic target for schwannoma and other merlin-deficient tumours. ●

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