Bone morphogenetic protein signaling as novel therapeutic target in pheochromocytoma

Andrea Richter, Ines Leinhäuser, Misu Lee, Ines Höfig, Natasa Anastasov, Falko Fend, Tonino Ercolino, Massimo Manelli, Anne-Paule Gimenez-Roqueplo, Mercedes Robledo, Ronald R. de Krijger, Felix Beuschlein, Michael J. Atkinson and Natalia S. Pellegrata

Background
MENX is a spontaneous multiple endocrine neoplasia-like syndrome in the rat, which is caused by a biallelic germline mutation in the Cdkt10 gene, encoding a very unstable mutant p27 protein (Figure 1C) [1]. MENX predisposes, among other neoplasias, to the development of bilateral pheochromocytomas (PCC) with complete penetrance (Figure 1) [2]. Gene expression profiling of MENX rat PCCs identified the growth factor BMP7 (bone morphogenetic protein 7) as highly expressed in tumors versus normal adrenal medulla (Figure 1D) [3]. Previous work demonstrated that upregulation of BMP7 enhances proliferation, migration and invasion of PCC cells. In primary rat PCC cells BMP7 expression sustained cell viability. In PCC BMP7 signals through the PI3K/AKT/mTOR pathway and integrin β1 [4]. The small molecule antagonist DM1H is a second-generation analog of dorsomorphin and inhibits most selectively BMP type I receptors (BMPR-I) (Figure 2) [4].

Aim

The aim of the project was to assess the role of BMP7 in PCCs of MENX rat and human PCC patients and to explore the effect of the small molecule compound, DM1H (BMPR-I analog), in BMP7-mediated PCC tumorigenesis in vitro and ex vivo.

Experimental Plan

We used cell lines such as MPC (mouse PCC) and its aggressive derivative MTT, both with high levels of Bmp7 and primary rat PCC cells with high levels of BMP7. To evaluate the effect of blocking Bmp7 signaling on PCC cells we treated these cells with DM1H, which selectively inhibits BMPR-I. In vitro assays assessing proliferation (MTT) and migration (Boyden chambers) were then performed. Additionally, we established an ex vivo system based on the rotary cell culture system from Syntecell-Cellon. With this system tissues will be cut in small pieces right after dissection of MENX rats and cultured ex vivo under DM1H treatment.

Results

The small-molecule BMP antagonist DM1H highly selectively inhibits BMP type I receptors, but no other off-target receptors [4]. To verify whether blocking BMP receptor signaling might be a potential strategy for targeted therapy of PCC, we treated MTT cells (high endogenous Bmp7 levels) with DM1H and then figures out a significantly suppressed MTT cell proliferation (Figure 3A), and even more strongly inhibited cell migration (Figure 3B). Concurrently, we observed a dose-dependent downregulation of the expression of P-Smad1/5/8 and integrin β1, both readouts of active BMP signaling in PCC cells, as well as P-AKT (Figure 3C). Next, we determined a decreasing effect of DM1H on rat primary PCC cells (high endogenous Bmp7 level) (Figure 3D). Furthermore, we investigated the effect of DM1H in PCC tissues of MENX affected rats ex vivo using a rotary cell culture system. We could show a reduction of BMP signaling downstream targets (P-AKT and P-S6) at the protein level by western blotting (reduction of around 20%) and by IF staining (P-Smad1/5/8) (Figure 4).

Conclusions

The Bmp pathway represents a novel therapeutic target in PCC! DM1H, a BMP receptor antagonist elicits anti-proliferative and anti-migratory responses in PCC cells with active BMP signaling in vitro and ex vivo. Future studies will address DM1H effects on PCC in vivo.

References
[1] Pellegrata, Quintana-Martinez et al., PNAS USA, 2006

Institute of Pathology

Endocrine tumours and neoplasia
Andrea Richter