ICGEB International Centre for Genetic Engineering and Biotechnology

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SEMINAR PROGRAMME 2017

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"Novel anti-angiogenic compounds based on modulation of VEGF splicing"

Host: E. Buratti

Through alternative splicing of the terminal exon in the VEGF-A gene, two functionally distinct isoform families are generated. Use of the canonical proximal 3' splice site results in the expression of the pro-angiogenic VEGF-Axxx isoforms, whereas use of the distal 3' splice site results in anti-angiogenic VEGF-Axxxb expression. With the use of a splicing sensitive fluorescent reporter (SSFR) designed to mimic VEGF-A exon 8 splicing, we are able to screen for novel compounds that switch VEGF-A splicing to increase the therapeutic VEGF-Axxxb isoform. A bichromatic SSFR was engineered to mimic VEGF-A exon 8 splicing. The reporter (termed VEGF8ab) consists of the endogenous VEGF-A intron 7 and required parts of exon 8, followed by the coding sequences for two fluorescent proteins. Depending on which 3' splice site is used, a different fluorescent protein is expressed. Therefore red fluorescent protein (RFP) denotes the pro-angiogenic VEGF-Axxx isoforms, and green fluorescent protein (GFP) denotes the anti-angiogenic VEGF-Axxxb isoforms. This splicing reporter allows VEGF-A alternative splicing to be studied both in vitro and in vivo. The VEGF8ab SSFR was first transfected in to HEK293 and PC3 cells in a stable manner, and then validated using treatments with growth factors and small molecule inhibitors known to switch VEGF-A splicing. Following successful validation, a large screen of 1280 pharmacologically active compounds (LOPAC library) was performed using several screening methods to identify nine hit compounds (named ESSOs) that switched the alternative splicing pattern, therefore decreasing the RFP/GFP ratio. Further experiments were carried out to confirm that several of the compounds switched the endogenous splicing pattern of VEGF-A to increase the VEGF-Axxxb isoforms at both the mRNA and protein level. Additionally, treatment of tumors and Matrigel plugs with these compounds resulted in decreased tumor growth and vessel density due to anti-angiogenic activity.

We have also engineered a transgenic mouse harbouring the VEGF8ab SSFR into the ROSA26 locus. Preliminary data from surveying several tissues in this mouse model confirms recent findings regarding VEGF-A splicing; e.g. in skeletal muscle it has been reported that VEGF-A165b levels are high and indeed we see an abundance of GFP (corresponding to VEGF-Axxxb) in skeletal muscle. We are using this mouse model to evaluate the effect of the ESSO compounds on VEGF splicing in vivo.

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