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Agonist Activity Of $VEGF_{165}b$ In HEK 293 Cells Expressing The Human VEGF Receptor 2

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Vascular endothelial growth factor (VEGF) is an agonist at VEGF receptor 2 (VEGFR2) associated with angiogenesis (1). $VEGF_{165}b$ is a splice variant of this protein that has often been described as an inhibitory isoform or weak partial agonist with anti-angiogenic activity (1,2). Here we have compared the agonist activity of $VEGF_{165}a$ and $VEGF_{165}b$ in HEK 293 cells expressing human VEGFR2.

HEK 293 cells expressing VEGFR2 (wild type or containing an N-terminal NanoLuc® tag; Promega) and an NFAT (nuclear factor of activated T-cells) luciferase reporter gene, were cultured in Dulbecco's modified Eagle's medium (DMEM) plus 10% foetal calf serum. VEGFR2-NanoLuc® cells were grown on poly-d-lysine-coated 96-well white, clear-bottomed Greiner plates at a density of 25000 cells per well for 24h, then serum starved overnight before experimentation. VEGFR2 cells were grown to confluence in flasks before being seeded in a DMEM+0.1% BSA suspension of 40000 cells per poly-d-lysine-coated well. All cells were then pre-incubated with DMEM+0.1% BSA ± cediranib (a tyrosine kinase inhibitor, final assay concentrations: $1x10^{-11}$M to $1x10^{-7}$M) before a further 5h incubation with $VEGF_{165}a$ or $VEGF_{165}b$ (final assay concentrations: $1x10^{-12}$M to $3x10^{-8}$M). For VEGFR2 cells, luciferase activity was measured using the One-Glo™ Luciferase Assay System (Promega) according to manufactures instructions. In the case of VEGFR2-NanoLuc® cells, medium was aspirated from the cells prior to addition of 50µl DMEM plus 50µl ONE-Glo™ reagent. Luminescence was measured on a TopCount NXT plate reader.

In both cell lines, $VEGF_{165}a$ and $VEGF_{165}b$ produced a concentration-dependent increase in luminescence, with $VEGF_{165}b$ producing a lower maximal response compared to that obtained with $VEGF_{165}a$ (Table 1). Cediranib inhibited the response to 3nM $VEGF_{165}b$ in a concentration-dependent manor (Table 1). The pIC_{50} values obtained for cediranib were similar to those obtained with 1nM $VEGF_{165}a$ in VEGFR2-NanoLuc® (9.33 ± 0.19, n=6) and VEGFR2 (3) cells (9.13 ± 0.01, n=5).

Cells	$VEGF_{165}a$ (pEC_{50})	$VEGF_{165}b$ (pEC_{50})	$VEGF_{165}b$ Maximal response (% of response to 30nM $VEGF_{165}a$)	Cediranib (pIC_{50}) (3nM $VEGF_{165}b$ as agonist)
VEGFR2-NanoLuc® (n=6)	9.87 ± 0.09	9.25 ± 0.06	63.2± 4.8 %	9.32 ± 0.11
VEGFR2	9.76 ±	9.21 ±	62.1 ± 1.2 %	9.38 ± 0.07

(n=5)	0.08	0.08		

Table 1. Concentration-response parameters for $VEGF_{165}a$ and $VEGF_{165}b$.

These data show that $VEGF_{165}b$ is a partial agonist at VEGFR2 in HEK 293 cells expressing an NFAT reporter gene. These data, taken together with previously published work (1,2), raise the possibility that the effect of $VEGF_{165}b$ on signalling may vary between cell types and with expression level of VEGFR2.

1. Woolard J *et al.* (2004). Cancer Res. 64: 7822-7835.

2. Suarez SC *et al.* (2006). Cell Mol Life Sci. 63: 2067-77.
3. Carter JJ *et al.* (2014). This meeting.