New angiogenesis targets

By Lev Osherovich, Senior Writer

An international collaboration among researchers at the **Karolinska Institute**, **Discovery Genomics Inc.** and **AngioGenetics AB** has produced a set of previously unknown and potentially druggable targets in

angiogenesis along with several matching antiangiogenic compounds.¹

AngioGenetics was acquired by **BioInvent International AB** in 2007, and BioInvent now holds joint IP rights to the discoveries along with Discovery Genomics. The discoveries are the result of a 2002 partnership between AngioGenetics and Discovery Genomics.

The study, which combined a genetic screen with a chemical one, was published in *Chemistry & Biology* and was led by Mats Hellström, senior scientist at Karolinska and senior principal scientist at BioInvent.

Although genetic and pharmacological screens for angiogenesis targets have been done before, Hellström said the team's effort is the first to combine the two approaches to link compounds to targets.

"To identify new angiogenesis targets, we created a cDNA library of endothelial genes and screened for their effect in zebrafish," said Hellström. "We then screened compound libraries for effects on those pathways."

Table 1. New angiogenesis targets and inhibitors. In a pharmacogenetic screening study, Kalén *et al.* have identified proteins needed for angiogenesis in zebrafish. Below are the human homologs of the genes identified by the study along with their biological function. A parallel small molecule screen identified compounds that could potentially mimic the effect of knocking down some of these genes; the compounds are also listed below.

Gene symbol	Name	Function	Small molecule mimics identified
ALOX5AP	Arachidonate 5-lipoxygenase-activating protein	Converts arachidonic acid to hydroperoxyeicosatetraenoic acid (HPETE), a precursor to leukotriene A4	Nimesulide and budesonide are small molecules that block steps in prostaglandin and leukotriene synthesis. Nimesulide is a generic NSAID already known to inhibit angiogenesis. Budesonide, an anti-inflammatory, is marketed as Pumicort by AstraZeneca plc (LSE:AZN; NYSE:AZN)
CTSZ	Cathepsin Z	Protease; homologs implicated in cancer, stroke and Alzheimer's disease (AD)	
FZD6	Frizzled homolog 6	G protein-coupled receptor for Wnt; signal transduction	
GNB2	Guanine nucleotide binding protein- β polypeptide 2	G protein subunit; signal transduction	
HEXB	Hexosaminidase B	Lysosomal protein involved in ganglioside degradation	
KIAA1274	KIAA1274	Protein tyrosine phosphatase (predicted by homology)	
P2RY5	Purinergic receptor P2Y G protein-coupled 5	G protein-coupled receptor for extracellular purine; signal transduction	
PPAP2A	Phosphatidic acid phosphatase type 2A	Converts phosphatidic acid to diacylglycerol, a precursor to prostaglandins and leukotrienes	Nimesulide and budesonide
PPIH	Peptidylprolyl isomerase H	Protein folding	
PPP1CA	Protein phosphatase 1 catalytic subunit- α isoform	Protein serine/threonine phosphatase	Endothal, a generic herbicide; cantharidin and cantharidic acid, insect-derived toxins with <i>in vitro</i> antitumor activity
PPP1CC	Protein phosphatase 1 catalytic subunit- γ isoform	Protein serine/threonine phosphatase	Endothal, cantharidin and cantharidic acid
PPP4C	Protein phosphatase 4 catalytic subunit	Protein serine/threonine phosphatase	Endothal, cantharidin and cantharidic acid
RAB11A	RAB11A	Small GTPase involved in secretion and protein transport	
RAB5C	RAB5C	Small GTPase involved in secretion and protein transport	
RALB	V-rat simian leukemia viral oncogene homolog B	Small GTPase involved in signal transduction	
SAT1	Spermidine/spermine N1-acetyltransferase 1	Enzyme that catalyzes the acetylation of polyamines	

The team initially used microarrays to identify cDNAs expressed in mouse vascular tissue. From an initial count of 150, the researchers winnowed the list down to 50 druggable candidate genes that were also present in zebrafish. The team then knocked down the expression of these genes in zebrafish embryos using morpholinos, a type of antisense molecule, and looked for changes in vascular development.

“This has not previously been done for such a complex vertebrate system,” said Hellström.

The team found that knockdown of 16 of these genes led to vascular defects that were not seen following treatment with control morpholinos. Among the 16 were 2 genes involved in prostaglandin biosynthesis and 4 previously uncharacterized phosphatases (see **Table 1**, “**New angiogenesis targets and inhibitors**”).

In parallel, the researchers screened a library of 1,280 pharmacologically active compounds for small molecules that caused vascular defects and came up with 28 compounds that inhibited endothelial sprouting but didn’t disrupt the behavior of nonvascular cells.

Among these compounds were two inhibitors of prostaglandin synthesis and a trio of broadly acting phosphatase inhibitors.

Hellström said that because prostaglandins have previously been linked to angiogenesis,² his team was more interested in exploring the phosphatases. He thinks the phosphatase inhibitors are likely to be acting on one or more of the phosphatases identified in the genetic screen.

“This has not previously been done for such a complex vertebrate system.”

**—Mats Hellström,
Karolinska Institute**

Indeed, Hellström’s team found that the specific defects in vascular morphology caused by knocking down the three phosphatase genes were mimicked by treatment with one of the phosphatase inhibitors.

The next step is to test the role of the three phosphatases in mouse models of tumor-associated angiogenesis.

“Even though phosphatases aren’t yet sufficiently validated as targets, we think they’re very interesting,” said Hellström.

He noted that phosphatases are intracellular proteins and so are not likely to be targetable by antibody-based therapeutics, which is BioInvent’s main focus. As a result, pending patents targeting the proteins identified in the study are available for licensing from BioInvent. Discovery Genomics did not comment on the study.

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