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Title: ANGIOGENIN AND ENDOTHELIAL CELLS.

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1 Angiogenin is a potent blood vessel-inducing polypeptide that has
2 a unique ribonucleolytic activity. Initially identified from tumor cells
3 conditioned medium through its ability to induce neovascularization in
4 the chick embryo chorioallantoic membrane assay [1], angiogenin has
5 also been purified from normal plasma [2] which suggested that it might
6 be involved in endothelium homeostasis. Angiogenin is highly
7 homologous to pancreatic ribonuclease A with 68% amino acid sequence
8 homology and conserved essential active site residues [3]. However,
9 angiogenin is inactive in standard ribonuclease assays but has a
10 ribonucleolytic specificity toward ribosomal and transfer RNAs [4]. This
11 enzymic activity might be relevant to the process of angiogenesis since
12 a functional active site seems necessary for the expression of its
13 angiogenic property [5], however, it appears not to be sufficient [6].

14

15 **Interactions of angiogenin with endothelial cells**

16 Using recombinant human angiogenin produced by Rhône-Poulenc Santé
17 and shown to be angiogenic, in vivo, both in the chick chorioallantoic
18 membrane and the rabbit cornea [7,8], we have demonstrated the
19 presence of high affinity binding sites on endothelial cells from calf
20 pulmonary artery (CPAE), bovine aorta, cornea and adrenal cortex
21 capillary that could not be detected on Chinese hamster lung fibroblasts
22 [9]. Receptors have also been reported on bovine brain capillary
23 endothelial cells [10]. The interactions on CPAE cells displayed an
24 apparent dissociation constant in the nanomolar range and involved
25 about 200,000 angiogenin molecules per cell [9]. This number, that
26 varies with the cell density, does not necessarily represent a number of
27 receptors. However, it includes receptors since angiogenin, added to
28 cultured cells, triggers off a series of intracellular events in
29 endothelial cells such as a transient increase in 1,2-diacylglycerol [11]
30 and activation of phospholipase A₂ [12], and in vascular smooth muscle
31 cells, activation of phospholipase C and a rapid cholesterol
32 esterification [13]. Besides, angiogenin might modulate mitogenic
33 stimuli [10,14].

34 Angiogenin has also been shown to interact with low-affinity/high
35 capacity specific binding sites on CPAE cells as well as on extracellular

1 matrix. This second family of interactions, with an apparent
2 dissociation constant of $0.2 \mu\text{M}$, involved millions of molecules [9].

3 An angiogenin-binding protein has been recently identified on bovine
4 endothelial cells as a dissociable cell-surface component of 42-KDa
5 released by treating the cells with heparan sulfate [15].

6 7 **Inhibition of angiogenin properties by Placental Ribonuclease** 8 **inhibitor**

9 Placental ribonuclease inhibitor (PRI) is a tight binding inhibitor of
10 both the ribonucleolytic and angiogenic activities of angiogenin [16]
11 with an inhibition constant value, K_i , in the femtomolar range [17]. This
12 inhibitor also abolishes angiogenin binding to CPAE cells [9]. However,
13 PRI is an intracellular regulatory protein and although immunoreactivity
14 has been detected in normal human plasma [18], there is not yet any
15 experimental evidence for angiogenin/inhibitor circulating complexes.

16 17 **Effects of copper metal ions on angiogenin binding to** 18 **endothelial cells and to ribonuclease inhibitor**

19 Divalent copper, a modulator of angiogenesis [19], has been shown to
20 induce a severalfold increase in specific cell-bound angiogenin and to
21 reverse PRI-induced inhibition of angiogenin binding to CPAE cells [8,9].
22 Furthermore, divalent copper metal ions inhibits angiogenin
23 ribonucleolytic activity towards tRNA substrate [20].

24 25 **Discussion**

26
27 The variety of copper interactions with angiogenin properties is
28 indeed puzzling. It might reflect a tight regulation of angiogenin
29 functions necessary to balance its angiogenic potency with its high
30 intravascular concentration. Copper also interacts with heparin and the
31 angiogenic growth factor FGF [21], and appears, from other reported
32 data, to modulate angiogenesis somehow [19]. At this point, it is worth
33 noting that copper is required for other fundamental events such as
34 central nervous system development, bone and connective tissue
35 biosynthesis, electron transport and antioxidant protection where it

1 acts as a prosthetic group [22]. Because of angiogenin peculiarities,
2 studies on its mechanism of action should lead the way to a better
3 knowledge of the biochemical events involved in angiogenesis.

4
5 In summary, modulation of angiogenin functions might involve
6 interactions with cell-surface receptors, binding protein and
7 extracellular matrix components, tight-binding inhibition of both its
8 ribonucleolytic activity and cell binding by ribonuclease inhibitor under
9 the overall influence of divalent copper, a modulator of angiogenesis.

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