

Summary

Human angiogenin (hAng) is a potent angiogenic factor, playing central roles in many physiological and pathological states including the growth and establishment of human tumors. hAng exerts many crucial biological functions, inducing cell growth and survival. hAng presents ribonucleolytic activity which is significantly lower than that of RNase A, but essential for its angiogenic activity. The extracellular hAng activates many signal transduction pathways, and it is also implicated in the extracellular matrix degradation, promoting thus cell migration and invasion. Under growth conditions hAng accumulates in the nucleus/nucleoli promoting the mRNA transcription and rRNA transcription/processing, while under stress conditions it accumulates in the cytoplasm where it forms tiRNAs leading to the inhibition of translational initiation. hAng has been validated as a pharmaceutical target inasmuch as the control of angiogenic activity offers a means to treat cancer.

In the framework of the present Thesis, biochemical studies on the role of hAng were performed with the overarching goal to elucidate the biological functions of this protein. The crystal structure of hAng in complex with sulphate anions was determined to highlight the structure-function relationship. Kinetic studies revealed that ammonium sulphate shows a similar inhibitory potency for hAng, EDN and RNase A, however, the sulphate binding pattern is significantly different in hAng from that in RNase A and EDN, with the sulphate ions bound at the surface of the protein and not at the active site. This finding revealed a different structural mode for the inhibition of hAng compared to that of the other RNase A superfamily members. Structural analysis of the hAng-sulphate complex suggested a structural pattern for the binding of hAng to its natural RNA substrate.

The specificity of the enzymatic active site of hAng was studied, using low molecular weight nucleosides, as a strategy for the development of new anticancer agents. Since binding to the active site of hAng is impeded by the C-terminal segment, and the architecture of the active sites of hAng, RNase A, and EDN is conserved, a plausible strategy for the development of specific hAng inhibitors might be the usage of RNase A and/or EDN inhibitors as lead compounds. The inhibitory action of two series of modified nucleosides to RNase A and EDN has been studied

by biochemical studies and X-ray crystallography. The most potent compounds were also evaluated for their antiangiogenic potency *in vivo* by targeting hAng. One of the most potent inhibitors has been studied further for its ribonucleolytic inhibitory potency *in vitro*. However, the *in vitro* potency of the inhibitor did not seem to justify the strong *in vivo* inhibitory potency against hAng angiogenicity. NMR studies revealed that the primary binding site is the cell binding site of hAng, whereas the secondary is the active site of the enzyme. This is the first time that a dual inhibitor of hAng is reported, blocking both the entrance of hAng into the cell and the ribonucleolytic activity.

At last, significant details about the implication of hAng in many signaling pathways were highlighted by immunoprecipitation coupled mass spectrometry (MS) analysis of the cytoplasmic and nuclear extracts from an endothelial cell line. The MS analysis of three different biological replicates identified many statistically significant potential hAng-interacting proteins in the two cellular compartments. The multiprotein complexes formed by the proteins identified, revealed significant aspects of hAng on many crucial molecular pathways. Proliferating cell nuclear antigen (PCNA) and hAng were the two most statistically significant proteins. PCNA was co-immunoprecipitated with the hAng specific antibody in the cytoplasmic fraction, but not in the nuclear. The interaction between hAng and PCNA was validated through further immunoprecipitation and immunoblot studies, and subsequently, through immunocytochemical analysis for the co-localization of these two proteins in the specific compartment. Further studies are needed for the determination of the biological role of this specific interaction in the cytoplasm.