SUMMARY

Human angiogenin (hAng), an unusual member of the secreted ribonuclease family, is a potent angiogenic factor with a role in a variety of physiological and pathological conditions. hAng presents in normal human tissues and fluids, such as plasma, amniotic fluid, tumor microenvironment, and cerebrospinal fluid. It plays a major role in the growth and establishment of human tumors since it is a potent stimulator of new blood vessels through the process of angiogenesis. It is involved in each stage of oncogenesis, making it a diagnostic and prognosis cancer marker and a validated pharmaceutical target for drug development for human malignancies.

The Ph.D. thesis mainly focuses on the interactions of hAng with proliferating cell nuclear antigens (PCNA). PCNA, the eukaryotic DNA sliding clamp, was first shown to act as a processivity factor of DNA polymerase δ, which is required for DNA synthesis during replication. However, besides DNA replication, PCNA functions are associated with other vital cellular processes such as chromatin remodeling, DNA repair, sister-chromatid cohesion, and cell cycle control.

Molecular details of the hAng-PCNA interaction were determined by a combination of various biophysical methods. The two proteins were shown to interact directly, through immunoprecipitation (IP) studies of hAng with PCNA in vitro and their interaction was quantified by isothermal titration calorimetry (ITC), obtaining information on stoichiometry, enthalpy, entropy, and binding kinetics of the association. ITC experiments also revealed that the hAng-PCNA association is strong with a Kd value in the nanomolar range (Kd= 130 nM). Moreover, their interaction surface was mapped by NMR spectroscopy. Based on this information, we created a model of the 3D structure of the hAng-PCNA complex using docking and molecular dynamics simulations. The validity of the model was tested by mutating hAng residues Arg5 and Arg101, which are at the molecular interface of the two proteins and interact with PCNA residues Glu55 and Glu130, respectively. Thus, we generated a single and a double mutant of hAng, hAng R5E and R5ER101E, replacing the two arginines by glutamic acid. Subsequently, biophysical characterization of the interaction of each of the hAng variants PCNA by ITC experiments validated the model was since the hAng-PCNA interaction was converted from a strong association to a medium-strength association with Kd values of 218 nM and 1160 nM, for each variant, respectively.

Recent studies have shown that phosphorylation of serine-threonine residues enables hAng to evade cytosolic ribonuclease inhibitor (RI) and enter the nucleus. Among the serine-threonine residues of hAng, four are of special interest, Ser87, which is at the molecular interface of the RI-hAng complex, and a cluster of three residues near the NLS, Ser28, Thr36, and Ser37. To investigate whether these residues contribute significantly to the conformation of hAng, crystallographic studies of the mutants hAng S28AT36AS37A and hAng S28AT36AS37AS87A were performed, where these residues were replaced by alanine. The structural studies revealed that these mutations do not cause any significant change in the structure of hAng.