Opinion

Protein folding and tumour angiogenesis-do we know enough?

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The intrinsic property of a living system is to survive and reproduce, and to maintain those essential aspects, a cell requires continuous production and usage of energy that comes from food, which is either synthesized inside it or taken up from the outside. For each and every process that a living cell goes through, be it generating energy, adapting itself to the environment, replicating or simply growing in size, it requires a network of proteins. These protein molecules in the form of enzymes, transporters, structural or chaperones play crucial roles in cell survival. Most of the proteins to be functionally active need to be folded, except the intrinsically disordered proteins some of which are functional in spite of the disordered state.[1] Thus a stretch of amino acids when folds it and takes up a defined structure and conformation to become functional plays a crucial role in the survival of a cell. And therefore protein folding becomes a key phenomenon and has always been an intriguing question for scientists. Anfinsen and his coworkers did extensive work since 1950s for more than two decades, and showed that protein folding during renaturation is governed by the thermodynamic hypothesis and the native conformation is one in which the Gibbs free energy of the molecule in a particular environment is the least.[2–6] Protein folding being a highly complex and intricate phenomenon involves interesting physics.[7] Substantial work has been undergone in the last 50 years to understand the protein folding mechanisms.

Techniques like near and far UV circular dichroisms, intrinsic tryptophan fluorescence, NMR have been used to understand the protein conformations in solution. Equilibrium or kinetic folding and unfolding have been performed for enzymes as small as lysozyme[8] and as big as Malate synthase G.[9] Along with these, mass spectrometry is extensively used to identify and understand protein conformations in multiple studies.[10–13] ESI-MS with its unique characteristic of detecting non covalent interactions has been immensely useful in structural analysis of proteins.[14–17] Due to changes in the solvent accessibility and the gas phase basicity, the ionization of the protein molecules is affected in ESI-MS and thus this feature has been exploited to infer the compactness of these molecules.[18–22] The more compact a protein, it will have smaller charge as compared to its respective unfolded state[10,23,24] and thus would be shifted towards higher m/z on the spectrum. Further time-resolved electro spray ionization mass spectrometry (ESI-MS) with online pulsed hydrogen/deuterium exchange (HDX) has been used to monitor folding of small proteins like ubiquity, myoglobin.[25–27]

Another approach that is applied is the limited proteolysis of proteins and their analysis using CD or MS.[27–30] Partly folded states of lysozyme and lactalbumin family proteins were identified and molten globule state[27] or Apo and holo myglobins[29] were studied. Since these proteomic and computational approaches have greatly increased our understanding of the intricate protein folding mechanisms, the question we often ask how protein mis-folding alters the physiology of a cell and therefore may lead to various disease outputs. Among many pathological conditions, the one we discuss in this opinion is angiogenesis, which is the process of new blood vessel formation. Angiogenesis is a complex, step-wise process that happens in both normal embryonic development as well as postnatal pathological processes, such as cardiovascular disease, cancer, and diabetes. Aberrant blood vessel formation, in the retina and the choroid is a major cause of vision loss during severe eye diseases, such as age-related macular degeneration (AMD). Also, various solid tumours increase their own blood supply by increasing angiogenesis. Studies suggest a key role for the unfolded protein response (UPR) in regulation of angiogenesis, which is through regulating the secretion of pro-angiogenic cytokine, VEGF, and balancing endothelial cell survival and apoptosis. The unfolded protein response (UPR) include a complex set of signaling pathways activated upon accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), during ER stress.

The UPR get activated during angiogenic stimuli such as hypoxia and ischemia in the cell.[31] Also, the molecular chaperones that normally facilitate protein folding in the ER[31] have been found to be expressed in the retina and in retinal endothelial cells and are up-regulated by the UPR. For instance, the 70-kDa heat shock protein (Hsp70) is abundantly expressed in endothelial cells and acts as a key mediator of tumor angiogenesis by controlling endothelial cell survival, proliferation, and migration.[32] Similarly, the oxygen- regulated protein 150 (ORP150), an inducible ER chaperone, regulates VEGF transport and secretion and therefore tumor angiogenesis.[31] A link between UPR signaling and the angiogenic process is evident in diseases, such as cancer, retinal angiogenesis, and ischemic renal disease.[33] There are studies showing that glucose deprivations activates UPR and induces the angiogenic switch by increasing expression of proangiogenic factors (VEGF, IL-6, FGF-2, etc.) and a concomitant decrease in angiogenesis inhibitors (CXCL14, and CXCL10) in tumors.[34] Also, partially blocking UPR signaling by activating transcription factor 4 (ATF4) or silencing protein kinase RNA–like ER kinase (PERK) reduced the production of VEGF. *In vivo* the knockdown of PERK in tumor cells slows down tumor growth and decreases blood vessel density.[34] Collectively, knowledge about unfolded protein response in context of angiogenesis, and upstream downstream pathways could bring specific new targets, and therefore inhibiting those mediators of ER stress may lead for the development of new therapeutics to inhibit tumour angiogenesis.

Although computational approaches have greatly helped and increased our knowledge about protein folding and regulation of various diseases, still a lot needs to be done. For example studies about various intermediates that are formed during the folding pathway, and why is one path preferred over the other during renaturation? When the upr does come into play? are still being pondered upon. At this point, we came a long way in the area of protein folding, but do we know enough, and more precisely in disease context? The answer is it's never enough, we still know very little of the phenomenon that happens spontaneously in a living unit.

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Conflict of interest

The author declares no conflict of interest.

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