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INVITED EDITORIAL

Prostate Cancer

Intrinsically disordered proteins and prostate cancer: pouring new wine in an old bottle

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An inconvenient truth in urology is that despite decades of intense research, prostate cancer (PCa) has remained one of the most prevalent cancers and leading cause of cancer-related deaths in men, particularly in the industrialized world.[1] It is rather sobering to acknowledge that even with early diagnosis and treatment, the incidence and death due to the disease are almost paradoxically projected to increase in the coming decades.[2]

There are at least two major challenges facing urologists addressing PCa. The disease is slow-growing and a patient is more likely to die from other unrelated causes than from PCa. However, in a fraction of the cases, the disease can become "high-risk" (GS ≥8), that is, metastasize and potentially turn lethal.[3] Second, patients with the so-called "low-risk disease" (GS ≤6) are advised to follow the watchful waiting/active surveillance approach, although they are routinely monitored with the intention of avoiding treatment unless there is evidence of disease progression.[4–6] Despite this cautious approach, however, the disease progresses in a significant fraction of these patients and much to the chagrin of the urologist, they face an imminent danger of developing high-risk disease.[6] Unfortunately, reliable biomarkers that could discern high-risk PCa patients who are likely to progress to metastatic disease or discern low-risk patients in whom the disease is likely to progress are currently not available to the urologists. Furthermore, the controversy over the use of PSA and the perils of overdiagnosis has only muddied the water.[7]

Prognostic challenges aside, there are no therapeutic options that can be offered to patients with low-risk PCa. On the other hand, patients with high-risk disease are recommended surgery followed by androgen deprivation therapy (ADT). Notwithstanding the potential side effects of surgery and the failure of ADT due to the emergence of drug resistance in most patients, pursuant to an initial positive response,[8,9] the big questions are why and how cancer cells develop therapeutic resistance and how we address resistance in the future. Developing the so-called "next-gen" drugs to an old target (i.e., androgen receptor), even if they are more effective, is not likely to be a viable solution.[10–12]

Perhaps, the present state of affairs is due, at least in part, to old school ideas such as (1) cancer is highly deterministic - it is driven by mutations,[13,14] (2) proteins are highly ordered - structure defines protein function,[15] and (3) small molecules only fit into well-folded protein domains to affect their function; therefore, drug design can be "rational" (also referred to as structure-based drug design [SBDD]).[16] Thus, it is imminent that we need new thinking.

This Special Issue of the Journal approaches the problem with a *tabula rasa*. The central theme here is that proteins need not always be structured to be functional. In a series of articles contributed by leading investigators who employ a variety of techniques and tools from multiple disciplines such as cancer biology, biochemistry, biophysics, structural biology, and nonlinear dynamics, two main tenets are enunciated.

The first tenet addresses how intrinsic protein disorder plays a critical role in orchestrating complex protein-protein interactions in physiological processes and how dysregulation can lead to pathological consequences. Research over the past 15 years

has unearthed compelling evidence indicating that a large fraction of eukaryotic proteomes comprise proteins or significant regions within them that are intrinsically disordered.[17] Intrinsically disordered proteins (IDPs) or regions (IDRs) by definition lack a rigid structure at least *in vitro*. However, many IDPs are observed to undergo transition from disordered conformational ensembles to folded structures upon binding to a cognate biological target ("induced fit") or *a priori*, especially in response to post-translational modifications ("conformational selection").[18] Furthermore, some IDPs exhibit dynamic excursions and stochastically switch conformational states while still remaining disordered.[19] Thus, IDPs appear to represent proteins that are only marginally unstable and can be tipped to populate conformations to become functionally active. Such changes in the structural ensemble sampled by the IDPs are similar conceptually to the conformational (fold) switching events seen in some marginally stable ("metamorphic") folded proteins in response to mutation or environmental triggers that result in new functions of the same protein.[20] Thus, by increasing the functional repertoire of the same protein, Nature's main goal here apparently is to give proteomes maximum physiological plasticity without inefficiently expanding genome size. However, some IDPs have been shown to remain largely disordered even when they remain functional[21–23] and have been referred to as "fuzzy complexes."[24]

Regardless, IDPs are known to play critical roles in many cellular processes such as signaling, splicing and transcriptional regulation.[25,26] Furthermore, because of the enormous "structural" plasticity that is the ability of IDPs to populate different conformational ensembles, IDPs occupy key nodal (hub) positions in cellular protein

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interactions networks (PINs)[27–29] that adopt a scale-free architecture, and play an important role in channeling information flow within the system by regulating the network's structural and functional integrity.[30,31] However, because of their inherent ability to engage in "promiscuous" interactions when overexpressed,[32] IDPs can "rewire" PINs affording the system a robust degree of plasticity to adapt to environmental perturbations.

The article by Landau et al.[33] serves as a primer introducing the uninitiated reader to IDPs and highlights their importance in PCa, while the article by Russo et al.[34] discusses the consequences of dysregulation of IDPs in PCa. Kumar[35] focuses on steroid hormone receptors as a class of IDPs and highlights the role of their conformational dynamics in therapeutic targeting, while Monaghan and McEwan[36] focus on the role of intrinsic disorder in AR function, a key player in PCa, and illustrate how emerging therapies might target the NTD and its binding partners in the disease. Finally, Kulkarni et al.[37] discuss how PAGE4, a highly prostate-specific IDP that potentiates c-Jun transactivation represents an attractive target for developing novel therapeutics for "low-risk" PCa patients.

The second tenet attempts to illustrate how "structural" plasticity at the molecular level may modulate phenotypic plasticity at the cellular level. Living systems such as cancer cells are Complex Systems. That is, they consist of many diverse and autonomous but interrelated and interdependent components that are densely linked. They behave nonlinearly, that is they cannot be described by a single rule or variable rules and their characteristics are not reducible to one level of description, but depend on the emergent dynamics of the intricate interactions among many variables. Interestingly, Uversky has suggested that IDPs/IDPRs themselves can be formally defined as complex systems since they seem to obey major rules proposed to describe the behavior of complex systems. In fact, he alludes to the possibility that IDPs represent "edge of chaos" systems which operate in a region between order and complete randomness or chaos, where the complexity is maximal.[38]

Of note, complex systems have the unique ability to self-organize. Self-organization is a process where some form of global order or coordination, for example, phenotypic switching, arises out of the local interactions between the components of an initially disordered system for instance, IDPs. Indeed, as Kauffman eloquently stated in his profoundly influential book, The Origins of Order, "Complexity of biological systems and organisms might result as much from self-organization and far from equilibrium dynamics as it does from Darwinian natural selection."[39] Furthermore, self-organization is spontaneous and is often triggered by random fluctuations amplified by positive feedback.[40] Thus, even the "simplest" biological processes may not be fully understood by a merely reductionist approach and a dynamical systems perspective may be essential.

To demonstrate how the tools of nonlinear dynamics could help explain phenotypic plasticity in a self-organizing system, Mooney et al.[41] interrogate epithelial to mesenchymal transition (EMT). They point out that the key players driving phenotypic plasticity in PCa are IDPs and discuss how phenotypic plasticity at the molecular level may contribute to stochasticity in phenotypic switching at the cellular level by rewiring PINs. Further, using a cogent mechanism-based mathematical model, they also illustrate how EMT in PCa may occur due to events that are stochastic rather than merely deterministic in nature, and can therefore lead to distinct sub-populations that can co-exist and interact among themselves, adding another layer of complexity. The authors conclude that targeting IDPs may be a new strategy to develop novel treatments for PCa, especially advanced disease.

It is clear that despite declaring war on cancer by President Nixon more than four decades ago, we still do not have a good handle on what causes most spontaneous cancers in general and PCa in particular, much less, a cure for it. At the risk of sounding invidious, we hope that cancer biologists will reconsider conventional wisdom and welcome this new thinking. Furthermore, in light of the remarkably evolutionary conserved network properties of the IDPs,[42] we trust researchers will appreciate the role of intrinsic disorder and stochasticity in cancer. While genomic instability and mutations are the hallmarks of all cancers, recent studies suggest that it may not only be the occurrence of genetic variations, but the regulation of their expression that contributes to their biological and clinical significance.[43] Given the important role of stochasticity in specifying cell fate,[44] these observations underscore further the dire need for new thinking in cancer. Thus, we trust this series of articles will inspire a flurry of activity that, in the near future, will lead to safe and effective treatments to prevent as well as manage PCa. However, as the Cambridge economist John Maynard Keynes famously said, "The difficulty lies, not in the new ideas, but in escaping from the old ones."

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