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Review

# The structural network of inflammation and cancer: Merits and challenges

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ABSTRACT

Inflammation, the first line of defense against pathogens can contribute to all phases of tumorigenesis, including tumor initiation, promotion and metastasis. Within this framework, the Toll-like receptor (TLR) pathway plays a central role in inflammation and cancer. Although extremely useful, the classical representation of this, and other pathways in the cellular network in terms of nodes (proteins) and edges (interactions) is incomplete. Structural pathways can help complete missing parts of such diagrams: they demonstrate in detail how signals coming from different upstream pathways merge and propagate downstream, how parallel pathways compensate each other in drug resistant mutants, how multi-subunit signaling complexes form and in particular why they are needed and how they work, how allosteric events can control these proteins and their pathways, and intricate details of feedback loops and how kick in. They can also explain the mechanisms of some oncogenic SNP mutations. Constructing structural pathways is a challenging task. Here, our goal is to provide an overview of inflammation and cancer from the structural standpoint, focusing on the TLR pathway. We use the powerful PRISM (PRotein Interactions by Structural Matching) tool to reveal important structural information of interactions in and within key orchestrators of the TLR pathway, such as MyD88.



Proteins perform their functions via interactions; among these, interactions with other proteins are the most common, and they control cell life. Thus, there is no wonder that a major effort by the scientific community has targeted the modeling of protein–protein interactions. The availability of structural data relating to the mode of protein interactions is crucial to the understanding of how function is executed, and its control. When tiled together in the framework of the cell, it helps obtain the information flow from the extracellular space, through the membrane, the cytoplasm, and into the nucleus, to turn genes on and off. Signaling is not linear: pathways merge and diverge, signals integrate, and the proteins, which are the nerve centers of these pathway crossings, are frequently involved in cancer. Mutations deregulating these proteins are often responsible for turning on, and keeping on, entire pathways. They can also deregulate proteins that act as repressors of over-expression and activity.

The community has invested much effort in constructing pathways and in devising simple and clear ways of presenting them. The most common among these is the so-called nodes-and-edges representation, where nodes are the proteins and edges their interactions. This representation has been extremely useful since it essentially provides an overview of which proteins interact, in much the same way as a reference guide. At the same time, such pathway diagrams also suffer from limitations [1], since the information that they provide is incomplete, and does not allow in-depth understanding of the processes and their regulation. In contrast, structural networks which piece together the structures of individual proteins are much more powerful [2]. Structural networks allow understanding of how pathways cross through key hub proteins; which domains are involved in the interactions and which residues. They provide insight into the question of whether the

*Abbreviations:* TLR, Toll-like receptor; PRISM, PRotein Interactions by Structural Matching; IAP, inhibitors of apoptosis; ECM, extracellular matrix; ROS, reactive oxygen species; IBD, inflammatory bowel disease; NSAIDs, non-steroidal anti-inflammatory drugs; TGF-β, transforming growth factor-1 beta; PTEN, phosphatase and tensin homologue; VHL, von Hippel Lindau; $T_{reg}$s, regulatory T cells; MDSCs, myeloid-derived suppressor cells; TAMs, tumor-associated macrophages; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; PRRs, pattern recognition receptors; PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; HMGB1, high mobility group box-1; LRR, leucine-rich repeats; TIR, Toll/IL-1R; MyD88, myeloid differentiation factor 88; Mal, MyD88 adaptor-like; TRIF, TIR domain containing adaptor inducing interferon-β; TRAM, TRIF-related adaptor molecule; SARM, sterile α and heat-armadillo motifs; IRAK, interleukin-1 receptor-associated kinases; IKK, IκB-kinase; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IRFs, interferon regulatory factors; IFN-α, interferon alpha; IFN-β, interferon beta; RANK, receptor activator of NF-κB; Ubc13, ubiquitin-conjugating enzyme E2N; PTMs, post-translational modifications.

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pathways are controlled allosterically; how constitutive gain- and loss-of-function mutations can turn the protein on or off; and how parallel pathways can take over during drug resistance. Structural pathways are also very useful in drug discovery, because they can help forecast the global effects on the cell [3–6]; and they can help in selecting drug targets which are not the direct 'ailing' dysfunctional protein [7,8]. Such drugs exploit conformational ensembles [9,10]; they may also make use of network dynamics [11].

Constructing the structural network of major pathways in the cell is challenging: it entails putting together available structures (determined by crystallography, NMR, and high quality models) when data about their interactions exist in the literature, and when these data are missing, predicting them. It necessitates modeling the linear pathways as well as pathway integration and branching; it also necessitates predicting if the protein can act as a dimer – homo or hetero, and possible oligomerization modes. To grasp the enormity of the challenge, recall that hub proteins, such as p53, can interact with tens or hundreds of partners; Raf can work as a monomer and as a dimer; and EGFR has two dimeric states, symmetric (inactive) and asymmetric (active). However, the interaction surfaces are often unknown [1,12–14].

So why are structural pathways important? Structural pathways predict new interactions, not observed in 'classical' pathway maps; in particular those involving scaffolding proteins; they allow identification of parallel pathways in drug resistant mutants; they may discover positive feedback loops, altering core processes, as in the case of inhibitors of apoptosis (IAP) [15]. They also provide interaction details, which allow identification of oncogenic mutations; help drug discovery; and allow assembling multi-subunit signaling complexes, such as the key complex MyD88.

A current goal in our lab is to take steps toward accomplishing the overarching task of modeling protein interactions in cancer-related pathways and the cancer network in the cell and mapping oncogenic mutations associated with these. Within this framework, here we aim to provide an overview of inflammation and cancer from this structural standpoint. Below, we first provide a broad description of the inflammation and cancer link that we aim to model, and some examples as they relate to this link. We then explain the challenge in modeling protein interactions on a large scale, particularly when seeking to also detect new interactions, which are not known a priori. We follow by explaining PRISM [16,17], the method that we use to model these pathways, its advantages and shortcomings.

Computational structural biology helps experiment by providing more complete information and leads. It is our belief that a complete map of key cellular pathways with structural data - including multimolecular associations of adaptor proteins - is critical to fully understand cancer cell biology with the abnormality originating from deregulation of signaling pathways.

## 1. Inflammation and cancer

Inflammation by innate immunity, which is required to fight microbial infections, heal wounds, and maintain tissue homeostasis, can lead to the hallmarks of cancer [18–20]. Several recent studies suggested that inflammation has an important role in all phases of tumor development, including tumor initiation, tumor promotion, invasion, metastatic dissemination, and evading the immune system [18,19,21]. Inflammation causes cellular stress and may trigger DNA damage or genetic instability [21], and chronic inflammation can contribute to primary genetic mutations and epigenetic mechanisms that initiate malignant cell transformation [20–22]. Tumor-promoting effects of inflammation alter tissue homeostasis, predisposing individuals to cancer [21,23,24]. The connection between inflammation and cancer has been established by Wirchow already in 1863, who noticed that cancers originate at sites of chronic inflammation and tumors have "lymphoreticular infiltrate" [20,21,25,26]. Inflammation establishes a tissue microenvironment, which tolerates tumor growth and metastasis by setting immunosuppressive mechanisms [21]. Therefore, inflammation not only induces carcinogenesis but also makes immune cells incapable of destroying tumor cells. A key initiator of a major pathway leading to inflammation is the TLR pathway (displayed in Fig. 1).

Inflammatory cells supply growth factors to maintain proliferation, survival factors to escape from apoptosis, pro-angiogenic factors and extracellular matrix (ECM) modifying enzymes that enable angiogenesis, invasion and metastasis [18]. Inflammatory cells can also secrete reactive oxygen species (ROS) that induce mutations, lead to failure of DNA repair, activation of oncogenes, and ultimately cancer [18,20,21]. ROS further activates inflammatory genes and takes part in tumorigenesis which is regulated by c-MYC, K-Ras, and Wnt signaling pathways [18].

The first evidence that connected inflammation with cancer was that inflammatory diseases such as inflammatory bowel disease (IBD) increase cancer susceptibility [27]. Additional evidence comes from the tumor microenvironment, which has inflammatory cells, cytokines and chemokines. In addition, long term administration of non-steroidal anti-inflammatory drugs (NSAIDs) leads to a decrease in the number of relapses and newly acquired tumors [18]. Moreover, pathways of inflammation function downstream of oncogenic mutations (MYC, RAS, BRAF, and RET), suggesting that these oncogenic mutations lead to activation of an inflammatory pathway [28]. Last but not least, inhibiting inflammatory cytokines and chemokines such as TNF-$\alpha$, IL-1$\beta$, or essential transcription factors, such as NF-$\kappa$B and STAT3, decrease the appearance of cancer [24]. Most of these proteins that connect inflammation and cancer are also members of TLR-pathway.

The TLR pathway in Fig. 1 is presented in the classical node-and-edge form. As we noted above, such pathway diagrams are informative and have been enormously useful in compiling and laying out the interaction maps. As can be seen in Fig. 1, they provide the interconnectivity between the proteins and outline the major signaling flow. Nonetheless, the information that they are able to provide is incomplete. Structural pathways can fill in gaps; add missing proteins and interactions, and provide the interaction details, which can help in figuring out parallel pathways, and the conformational mechanisms that control them. These are important for understanding function under physiological conditions and oncogenic mutations and drug resistance in disease.

The link between inflammation and cancer appears to stem from two pathways [29], the intrinsic and extrinsic inflammation pathways, as shown in Fig. 2. Intrinsic inflammation is initiated by mutations that lead to activation of oncogenes and inactivation of tumor suppressors (tumor-promoter role) [24]. On the other hand, in the extrinsic pathway, infection or inflammation precedes cancer and increases the risk of cancer (tumor-initiator role) [24]. The similarity between cancer tissue and inflamed tissue involves angiogenesis and tissue infiltrating leukocytes, such as lymphocytes, macrophages and mast cells [18,19].

RAS, MYC, RET, and BRAF are among the oncogenes that stimulate inflammation [21]. Besides their roles in tumor initiation and promotion, oncogenes impact the relationship between the tumor and its microenvironment. The RAS-RAF signaling pathway is involved in most cancer types, and the activated proteins along the pathway induce the secretion of pro-inflammatory cytokines and chemokines [30]. Another oncogene, MYC, has a critical role in tissue remodeling of ECM [24,31]. RET, a tyrosine kinase, can be constitutively activated by mutations even in the absence of its ligands.