Book Chapter

Inflammasomes: Key Players in the Development of Cancer

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Innate Immune Response

Immunity refers to the resistance of the body against pathogenic microbes, their toxins, or other kinds of foreign substances through the ability to distinguish self from non-self. Immune responses can be broadly divided into innate and adaptive [1]. Innate immunity represents the first line of host defense against

pathogens. Effectors of innate immune responses include epithelial barriers in the skin and mucosae, their secretion of antimicrobial proteins, mucus and enzymes (e.g., lysozyme in tears), as well as phagocytes (monocytes, macrophages and neutrophils) and the complement system [2]. As a result of the activation of cell receptors specific for microorganism molecular patterns, innate immune cells are able to recognize diverse microorganisms such as bacteria, viruses, and fungi. This set of germline-encoded pattern recognition receptors (PRRs) has evolved to detect the presence of non-self through the binding of highly conserved microbial molecular features known as pathogen associated molecular patterns (PAMPs), which are essential for the survival of microorganisms and therefore difficult for the microorganism to alter. PRRs also recognize molecules released by damaged cells, so called danger associated molecular patterns (DAMPs), or perturbations induced by pathogens (patterns of pathogenicity) such as bacterial pore-forming toxins, perturbations of the cytoskeleton, and various types of cell stress. The engagement of PRRs leads to the activation of various inflammatory pathways that contribute to host defense. PRRs are expressed by several cell types involved in innate immune responses, such as monocytes, macrophages, dendritic cells (DCs), neutrophils and epithelial cells [3]. PRRs include Toll-like receptors (TLRs: transmembrane receptors), Nod-like receptors (NLRs: cytoplasmic sensors), RIG-I-like receptors (RLRs: cytoplasmic RNA helicases), and C-type lectin receptors (CLRs: transmembrane receptors) [2].

Inflammation is a normal physiological process which involves a well-organized cascade of changes within the living tissue in response to injury. Based on visual observation, inflammation is characterized by five main signs which are heat, redness, pain, swelling, and loss of function. These signs reflect increased blood flow, elevated cellular metabolism, vasodilation, release of soluble mediators, extravasation of fluids and cellular influx. Inflammation is a process that protects the host from invading pathogens or injury. The process dissipates after a short or long period of time, resulting in acute or chronic inflammation, respectively [4]. Inflammation requires a great amount of metabolic energy and can result in tissue damage and

destruction. Therefore, control mechanisms over the termination of inflammation are required [5]. Chronic inflammation is usually caused by persistent infections such as the microbes difficult-to-eradicate like Mycobacterium tuberculosis and Treponema pallidum, prolonged exposure to potentially toxic agents like silica, and monogenic and polygenic immune-mediated inflammatory diseases. Systemic chronic inflammation leads to disease complications such as diabetes mellitus, chronic kidney disease, autoimmune disorders and cancer [6].

A key signaling pathway that leads to acute and chronic inflammation is through the activation of the caspase-1 inflammasome which is assembled upon activation of certain nucleotide-binding domain, leucine-rich repeat containing proteins (NLRs), a cytoplasmic multimeric protein that causes the activation and the secretion of interleukins mainly Il-1b and Il-18 after being exposed to a stimulus [7].

Inflammasomes

Inflammasomes orchestrate pathogen defense and the repair process by activating the inflammatory response. They are formed after the detection of DAMPs and PAMPs by NLRs. Inflammasomes correspond to multi-protein complexes usually formed of two NLRs leading to the cleavage of the 17kDA pro-inflammatory cytokine IL-1b, from its 31kDa precursor, pro-IL-1B, via caspase 1 (Figure 1). This process also leads to activation of the IL-1 family member IL-18. Activation of the inflammasome is also associated with the onset of a form of cell death termed pyroptosis [8].

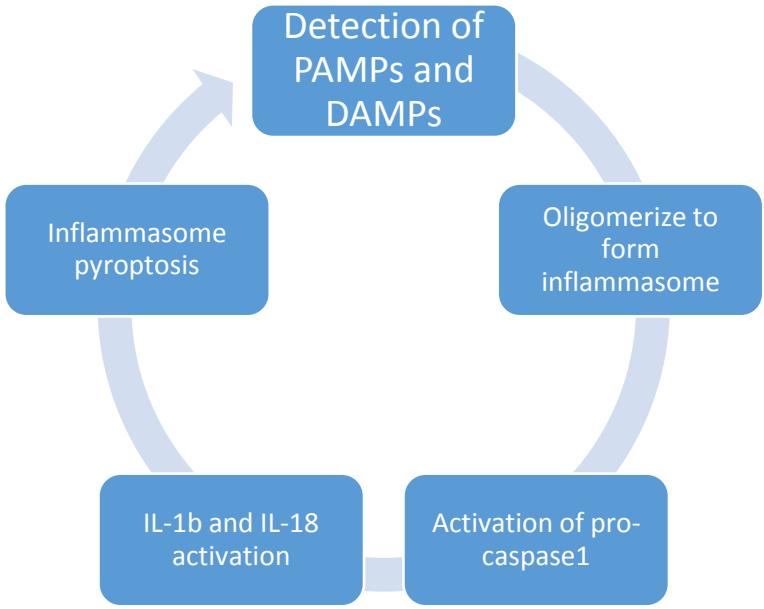

Figure 1: Pathway for inflammasomes activation after its stimulation. Once PAMPs and DAMPs are detected, inflammasomes are formed that in turns lead to pro-caspase1 activation. This results in IL-1b and IL-18 secretion and pyroptosis cell death.

The name inflammasome comes from the word inflammation, which reflects the function of the complex, and "some", which is from the Greek word for body, *soma*. The name also reflects similarities with the apoptosome, which triggers apoptosis (Zou et al., 1999). The inflammasome can recognize a wide variety of dangers, both internal and external. Endogenous signals that are known to activate the inflammasome include uric acid, ATP and potassium efflux [9]. On the other hand, external signals include stressors derived from a diverse range of conserved molecular motifs that are unique to bacteria, viruses and parasites, exogenous chemicals and ultraviolet light. The mechanism by which these signals are detected has yet to be fully elucidated for the majority of inflammasomes. The core structure of all inflammasomes is caspase-1, a variety of other proteins co-assemble with procaspase-1 to bring about its activation. Many, but not at all inflammasomes, have a member of the nucleotide-binding domain and leucine-rich repeat containing (NLR) gene

23 Like Family [10]. The production of IL-1B and IL-18 by the inflammasome is one of the first lines of defense against tissue damage and pathogen invasion. The inflammasome clinical importance exceeds infectious diseases, where its deregulation can lead to numerous inflammatory disorders [11]. Examples of diseases associated with inflammasome deregulation include Multiple Sclerosis, Alzheimer's disease, Parkinson's disease, Atherosclerosis and type2 Diabetes (Guo et al., 2015).

NLRPs

Nucleotide-binding domain leucine-rich repeat-containing receptors (NLRs) regulate innate immunity by activating inflammatory responses in a variety of biological systems following the recognition of pathogen- or disease-associated molecular patterns in the cell cytoplasm. Twenty-two NLRs have been identified in humans which presents the NLR family as a major class of intracellular PRRs. NLRs possess three different domains, an N-terminal death-fold domain that directly or indirectly engages caspase-1, a central nucleotide-binding or NACHT domain, and a C-terminal leucine-rich repeat (LRR) domain [8]. NLRs are classified based on their N-terminal domain. Once activated, NLRs induce a number of signaling pathways such as the pro-inflammatory NF-kB (for NOD1 and NOD2) and the caspase-1 inflammasome (for NLRC4, NLRP3 and NLRP1) pathways in addition to the induction of autophagy (NOD1, NOD2, and NLRC4) and cell death (NLRC4, NLRP3, and NLRP1). NLRs detect various DAMPs and PAMPs, where Nod1 and Nod2 recognize bacterial peptidoglycan specific structures, NLRC4 detects bacterial flagellin, and NLRP3 (inflammasome triggering protein) detects molecules like ATP, muramyl dipeptide (MDP) bacterial toxins, viral nucleic acids, b-amyloid fibrils and potassium efflux. Following the detection of PAMPs and DAMPs. NLRs activation leads to inflammasome assembly, through the recruitment of ASC adaptor protein (apoptosis-associated speck-like protein containing a CARD), ASC, that contains a pyrin domain (PYD) and a caspase activation and recruitment domain (CARD) allowing to bridge either a PYD or a CARD domain from the activated NLR and the CARD domain of pro-caspase-1 (pro-CASP1). Next, pro-

CASP1 undergoes proximity-induced auto-proteolysis to form an active enzyme (CASP1) that leads to the cleavage and activation of inflammatory cytokines (example: IL-1β and IL-18) and gasdermin D (GSDMD), the N-terminal resulting from this cleavage induces a pro-inflammatory form of programmed cell death known as pyroptosis [12].

IL-18 and IL-1b

Interleukin-1B (IL-1B) also known as catabolin, is a member of the interleukin 1 cytokine family of ligands, which also includes IL-1a, IL-18 and IL- 33. IL-1B is a pleiotropic cytokine that is involved in inflammation, cell growth, and tissue repair [13]. IL-1B is produced by blood monocytes but also by macrophages, dendritic cells and a variety of other cells in the body. IL-1B participates in the generation of systemic and local responses to infection and injury by generating fever, activating lymphocytes and promoting leukocyte infiltration at sites of infection or injury. IL-1B is believed to be the major mediator of inflammation in the periodic fever syndromes caused by mutations in NLRP family genes. It has been shown that treatment of the patients with an IL-1 receptor antagonist (IL-1Ra) or with anti-IL-1b neutralizing antibodies can improve their symptoms [14]. IL-18 induces IFN-b production and contributes to T-helper 1 (Th1) cell polarization. IL-18 also regulates Th2 and Th17 cell responses, in-addition to the activity of CD8 cytotoxic cells and neutrophils, in a host microenvironment-dependent manner and also boosts expression and production of certain cytokines, chemokines, and adhesion molecules (Wawrocki et al., 2016).

NLRP1

NLRP1 (nucleotide-binding domain leucine-rich repeat pyrin domain containing 1) was the first discovered PRR to form an inflammasome and with 1473 amino acids, human NLRP1 is the largest member of the family. NLPRs family members share three characteristic domains, the N-terminal death-fold domain or pyrin domain “PYD” that directly or indirectly engages caspase-1; the central nucleotide-binding or NACHT domain

made of "NAIP, CIITA, HET-E and TP-1" and the C-terminal leucine rich repeat LRR domain (Figure 2). NLRP1 also possesses two additional domains: (1) FIIND "function-to-find domain" that consists of ZU5 and UPA, which auto-processes NLRP1 into two polypeptide chains remaining non-covalently associated; (2) a C-terminal CARD domain signaling toward caspase-1 activation. Binding to a ligand causes NLRP1 self-oligomerization in a manner that is dependent on auto-proteolytic cleavage within the FIIND domain. Due to the poor conservation between mice and human, the molecular mechanisms for its activation and the resulting downstream events are still poorly understood. NLRP1 inflammasome stimuli can be divided into "direct activators" such as Anthrax lethal toxin and Shigella Flexneri that directly cause NLRP1 activation through degradation of the N-terminal PYD domain, and "indirect activators" such as Toxoplasma gondii and VbP the DPP8/9 inhibitor that cause disturbances within the cell that can be detected by NLRP1 [15]. It is worth to mention also that NLRP1 acts as sensor for virus infection by being stimulated by long dsRNA through binding it through its LRR domain [8]. NLRP1 is expressed mainly by keratinocytes and fibroblasts, and it is the only detectable inflammasome sensor in the human skin. Importantly, keratinocytes but not fibroblasts express a functional NLRP1 inflammasome. In that sense, not only do keratinocytes form the outer barrier of the skin but also play a role of immune cells by expressing molecules and peptides involved in immune responses. NLRP1 is regarded as the principal inflammasome sensor in human keratinocytes and UVB radiation induces its activation, which is believed to underlie the induction of sunburn. Moreover, gain-of-function mutations of ***NLRP1*** cause inflammatory skin syndromes. Thus, the detection of stress factors in the skin can lead to inflammation after NLRP1 activation and pro-inflammatory cytokine secretion. In addition, gain-of-function mutations lead to various skin disorders and increased risk for skin cancer. Therefore, pharmacological targeting of NLRP1 in epidermal keratinocytes represents a promising strategy for the treatment of patients suffering from NLRP1-dependent inflammatory skin disorders and cancer [16].

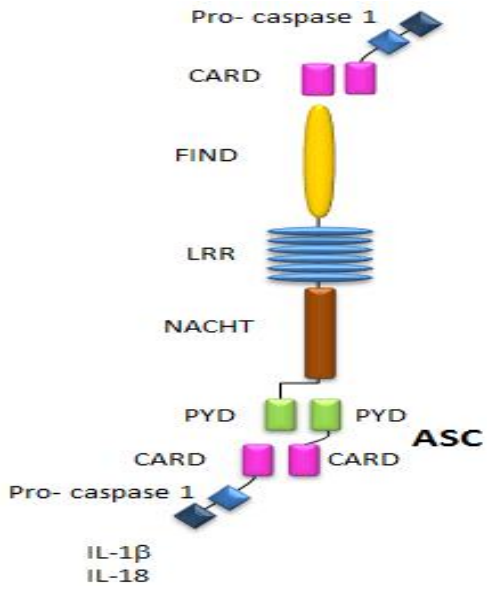

Figure 2: Schematic representation for the different domains of NLRP1. NLRP1 has three characteristic domains (PYD, NACHT and LRR) in addition to FIND and CARD domains.

NLRP1 Mutation

NLRP1 PYD domain interacts with the LRR domain to maintain NLRP1 as an inactive monomer. In some cases, a gain-of-function mutation on NLRP1 domains can lead to permanent activation and continuous secretion of interleukins in the absence of stimuli, thus leading to systemic chronic inflammation. When PYD or LRR domains are mutated, the auto-inhibitory mechanism is lost and NLRP1 becomes spontaneously activated triggering inflammasome assembly and thereby leading to pyroptotic cell death and IL-1 release from the initiating cells (Figure 3).

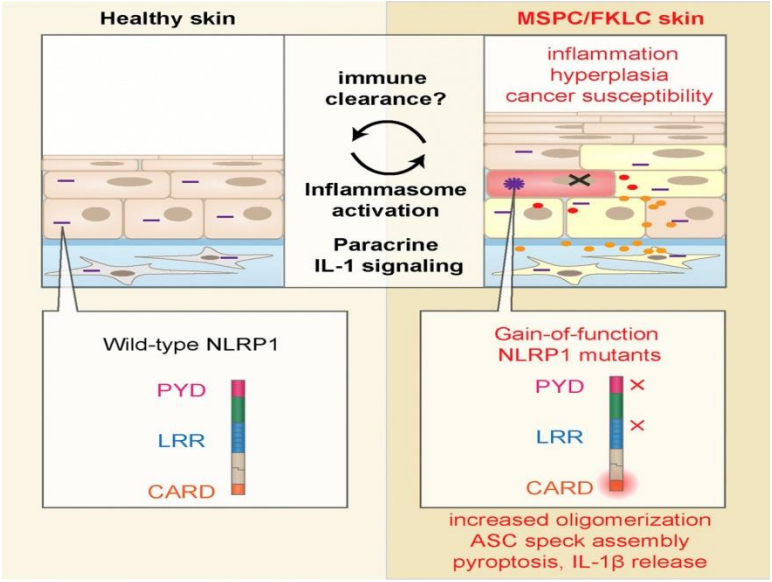

Figure 3: Gain-of-function mutations in the inflammasome sensor NLRP1 increase susceptibility to skin cancer and unmask unique regulatory auto-inhibition in the inflammasome [17].

IL-1 triggers the release of other inflammatory cytokines and growth factors such as TNFα and KGF from surrounding cells including neighboring keratinocytes and fibroblasts. A paracrine pro-inflammatory environment is created triggering epidermal hyperplasia and keratoacanthoma formation. Unresolved inflammation over years facilitates acquisition of additional oncogenic mutations and promotes malignant transformation toward SCC development. These gain-of-function mutations in Nod like receptors (NLRs) which activate inflammasomes leading to cytokine secretion cause systemic auto-inflammatory diseases. This has shed the light into the use of targeted anti-cytokine treatments that block interleukin signaling and help in the development of medicine in auto-inflammation [17]. The two most well-known skin disorders linked to NLRP1 mutations are MSPC "Multiple self-healing palmoplantar carcinoma" and the FKLC "Familial keratosis lichenoides chronica". The PYD domain is mutated in case of MSPC and the LRR domain is mutated in case of FKLC (Figure 4).

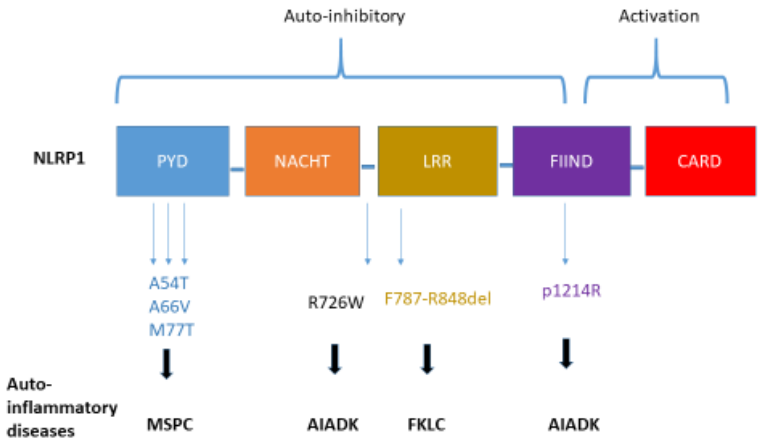

Figure 4: Different skin disorders associated with different mutations in different domains of NLRP1. Each domain of NLRP1 has a unique function and its mutation lead to different auto-inflammatory disease.

Inflammasomes and Cancer

During inflammation tissues become damaged and chemicals are released, causing white blood cells to secrete substances that stimulate cell growth resulting in wound repair. Inflammation ceases once the injury has healed, because it is a response to a stimulus and the removal of a stimulus should result in its abatement. In chronic inflammation, the inflammatory process may start even if there is no injury and persist for prolonged periods. Gain-of-function mutations on some of NLRP1 domains can lead to permanent activation of inflammasomes resulting in continuous secretion of interleukins especially IL-1β even in the absence of a stimulus as in the case of systemic chronic inflammation. Chronic inflammation is one of the most known hallmarks of carcinogenesis, allowing tumor growth and invasion. In contrast to NLRP3, NLRP1 has not been precisely analyzed. It plays a role as negative regulator of malignant melanoma cells apoptosis, where it binds caspase 1 and 9 in melanoma preventing their activation by other proteins and as result inhibiting their mediated apoptotic pathway. In that sense, NLRP1 overexpression promotes melanoma progression. In recent years, therapies targeting inflammasomes have been

widely improved to overcome human diseases such as cancer, where much attention has been paid to the development of compounds that inhibit the IL-1β signaling pathways as another approach for inflammasome inhibition in cancer treatment. Using IL-1 receptor antagonist (IL-1Ra) inhibits these effects thereby inhibiting the proliferation of skin carcinomas [18]. For example, Anakinra is a recombinant human IL-1 receptor antagonist and is regarded as a biological agent that blocks the inflammatory effects of IL-1 [19]. It was observed that its administration causes a dramatic improvement in hyperkeratosis which suggests that this treatment may regulate IL-1-induced keratinocyte hyperplasia and potentially reduce the risk of skin carcinomas in patients. Other examples of available treatments are Nidanilimab the ILR antibody, xilonix the IL-1α blocker and canakinumab the IL -1b blocker (Figure 5) [20]. It is also worth mentioning that some melanoma cancer cells gain drug resistance through a complex mechanism, in which nuclear factor-κB (NF-κB) and interleukin-1β (IL-1β) are critical contributors. Because NACHT, LRR and PYD domains-containing protein (NLRP) inflammasomes mediate IL-1β maturation and NF-κB activation, the role of inflammasome sensor NLRP1 in acquired drug resistance to temozolomide (TMZ) in melanoma was investigated [21]. Inflammasomes remain poorly understood with regards to their role in cancer development. Better understanding of their signaling regulation may help pave the way for future improvements in cancer prevention and treatment.

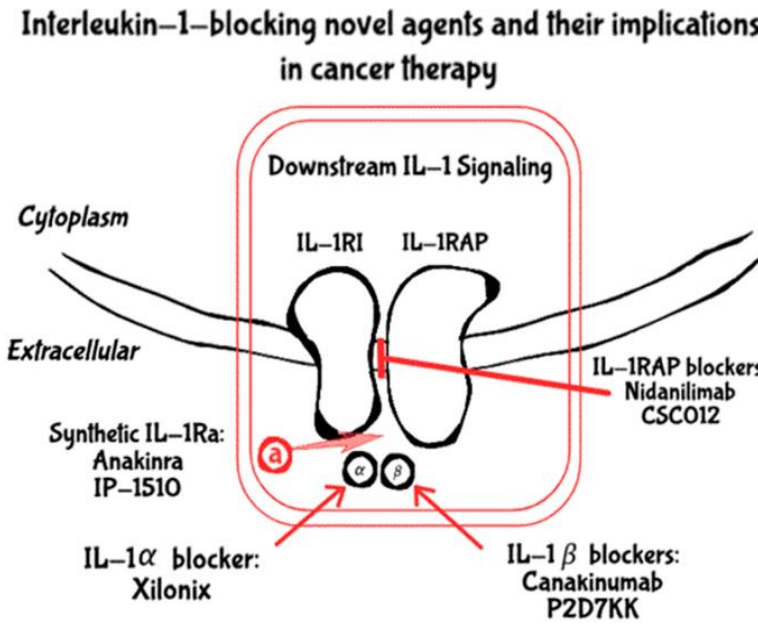

Figure 5: Novel IL-1 blockers used in clinical trails for cancer therapy. Xilonix and Canakinumab are IL-1 α monoclonal antibodies that bind to specific regions on their targets and prevent agonists binding to IL-1RI, thus inhibiting downstream signaling pathways. Nidanilimab and CSC012 are antibodies that prevent IL-1RAP from forming the heterotrimeric complex [20].

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