The Tumor-Intrinsic NLRP3-HSP70 Signaling Axis in Immune Evasion

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Contributor: Brent Hanks

The tumor-intrinsic NLRP3 inflammasome is a newly recognized player in the regulation of tumor-directed immune responses and promises to provide fresh insight into how tumors respond to immunotherapy.

Keywords: NLRP3 inflammasome ; Anti-Tumor Immunity ; HSP70 ; Adaptive Resistance ; Immunotherapy Resistance

1. NLRP3 in Cancer

The NLRP3 inflammasome has been implicated in promoting the progression of several malignancies by influencing the intrinsic invasiveness of the tumor while also promoting the process of epithelial-mesenchymal transition (EMT) [1][2][3][4][5][6][7][8][9][10]. Indeed, NLRP3 has been shown to be overexpressed by many cancers, while elevated levels of tumor NLRP3 expression have also been associated with an inferior clinical prognosis [5][11][12][13]. This is consistent with data showing *NLRP3* amplification across several solid tumor types, implicating *NLRP3* as a potential oncogene [14]. However, the underlying intrinsic pathways linking NLRP3 with tumorigenesis are still poorly described. Interestingly, one study has identified *NLRP3* exonic mutations associated with FLIP-mediated anti-apoptosis in ~16% of lung adenocarcinomas [15]. NLRP3 activation has also been suggested to contribute to myelodysplastic syndrome by triggering enhanced nuclear translocation of β-catenin and an increased expression of Wnt/β-catenin target genes in hematopoietic stem cells [16]. Still, it is unknown whether NLRP3 activation contributes to tumor de-differentiation via this same impact on the β-catenin signaling pathway in solid tumors. While pyroptosis has been shown to be suppressed in response to NLRP3 activation in certain tumor types, thus promoting tumor cell survival, it is unclear whether the development of this endpoint depends upon the specific stimulus [17]. Mechanisms determining the cell's fate of pyroptosis versus a state of inflammasome-dependent hypersecretion in tumors following NLRP3 activation would be expected to significantly alter the physiologic impact of this pathway [18].

In addition to its tumor-intrinsic properties, studies have described a role for NLRP3 in regulating the accumulation of immunosuppressive myeloid-derived suppressor cells (MDSCs) within the tumor microenvironment. This is exemplified by cancer-associated fibroblast (CAF) expression of NLRP3, which contributes to tumorigenesis and metastasis in several breast cancer models, including the syngeneic PyMT-derived mammary tumor cell lines, AT3 and Met-1, and the 4T1 mammary carcinoma cell line [19]. This study showed NLRP3 in CAFs to mediate IL-1β-dependent recruitment of both $CD11b^{+}Ly6C^{lo}Ly6G^{+}$ PMN-MDSCs and $CD11b^{+}Ly6C^{hi}Ly6G^{-}$ monocytic MDSCs into the tumor bed depending on the genetic background of the tumor model. These findings are in line with previous work implicating a role for host NLRP3 in suppressing the efficacy of a dendritic cell-based cancer vaccine by recruiting $CD11b^{+}Gr\text{-}1^{+}$ MDSCs into tumor tissues [20]. Further studies have similarly described the periodontal pathogen, *Porphyromonas gingivalis*, as a driver of colon cancer by promoting NLRP3-mediated accumulation of $CD11b^{+}$ myeloid cells into the colonic epithelium [21].

2. Tumor-Intrinsic NLRP3 and Its Regulation

While the regulation of the NLRP3 inflammasome has been well examined within various myeloid populations of the innate immune system, very few studies have investigated the tumor-intrinsic properties of NLRP3. It has remained unclear what biological processes NLRP3 may regulate in tumor cells and whether these processes may be different from its function in myeloid cell populations. In addition, it is not understood whether there are tumor-specific pathways that uniquely modulate NLRP3 function relative to the regulatory networks that have been shown to control NLRP3 activity in myeloid cells. However, there are newly available reports that have begun to provide some insight into NLRP3 regulation in cancers. A recent study found $Kras^{G12D}$ oncogene-driven myeloproliferation to be dependent upon NLRP3 inflammasome activation [22]. This work revealed $Kras^{G12D}$ induction of NLRP3 activation to rely upon RAC1-mediated accumulation of reactive oxygen species (ROS) in myeloid leukemia cells. This NLRP3 regulatory mechanism is similar to

what has been described for insulin-like growth factor-I shown to induce NLRP3 activation via ROS accumulation in HeLa cervical carcinoma cells [23]. Further studies have also demonstrated a pro-tumorigenic role for NLRP3 under the regulation of interleukin-1 receptor-associated kinase-1-mediated JNK1/2 phosphorylation in hepatocellular carcinoma cell lines as well as the control by estrogen receptor signaling in endometrial carcinoma cell lines and breast carcinoma cell lines [24][25][26]. Additional reports have described roles for microRNA-22 (miR-22), miR-135a, and miR-233 in negatively regulating NLRP3 expression while suppressing the progression of an oral squamous cell carcinoma model, a pancreatic cancer model, and a breast cancer model, respectively [27][28][29]. Future studies to uncover tumor-selective regulators of the NLRP3 inflammasome may allow for the development of pharmacologic agents that avoid impacting myeloid cell inflammasome activity and thus circumvent the development of potential off-target toxicities.

3. NLRP3 and Anti-Tumor Immunity

As opposed to its reported role in the innate immune system, NLRP3 inflammasome-mediated regulation of adaptive immune responses to cancer has been less well-described [30]. Prior work has supported a role for NLRP3 in tumor immunosurveillance, including studies showing the NLRP3 inflammasome to upregulate PD-L1 expression and suppress the generation of T cell responses in diffuse large B cell lymphoma while also driving metastatic progression in the B16/F10 melanoma model by inhibiting NK cell activity [31][32]. More recent studies have shown systemic pharmacologic inhibition of NLRP3 to increase effector $CD8^+$ T cells and suppress both $CD4^+FoxP3^+$ regulatory T cells and $CD11b^+Ly6G^+Ly6C^{lo}$ PMN-MDSCs in a transgenic *Tgfbr1/Pten* 2ccKO model of head and neck squamous cell carcinoma [33]. These data are also consistent with studies performed in a transgenic $p48^{Cre}$;$LSL\text{-}Kras^{G12D}$ model and an orthotopic $Pdx1^{Cre}$;$LSL\text{-}Kras^{G12D}$;$Tp53^{R172H}$ model of pancreatic cancer, which demonstrated NLRP3 signaling in macrophages to drive M2 polarization and suppress both the activation and infiltration of $CD4^+$ and $CD8^+$ T cells in pancreatic tumors [34]. This data is further in line with findings implicating macrophage NLRP3 signaling to enhance the migration and metastatic progression in models of both colorectal cancer and melanoma [35][36]. Additional studies have shown NLRP3 expression by MDSCs to mitigate against the anti-tumor properties of 5-fluorouracil chemotherapy in several preclinical tumor models by driving T_H17 development [37]. Notably, these effects were both found to be IL-1β-dependent. Indeed, many of the described pro-tumorigenic properties of NLRP3 have been attributed to its role in driving the expression of IL-1β by tumor-infiltrating myeloid cells. Studies have shown this process to support the recruitment of other myeloid cells to the tumor bed and promote tumor invasiveness and metastasis in an IL-1β-dependent manner [36][38]. Indeed, studies have implicated the NLRP3-mediated release of IL-1β in the induction of the IL-22 cytokine by $CD4^+$ T cells, a process observed to support tumor cell proliferation and growth [39]. Together, these data are consistent with the findings reported in the CANTOS clinical trial, which was originally designed to examine the impact of the IL-1β antagonistic antibody, canakinumab, on recurrent vascular events in patients with a prior myocardial infarction and persistently elevated C-reactive protein. Remarkably, a re-evaluation of this data revealed a significant decrease in lung cancer incidence (HR 0.33, $p < 0.0001$) and lung cancer mortality (HR 0.23, $p = 0.0002$) in patients treated with canakinumab relative to placebo [40].

While several studies have demonstrated IL-1β to generally promote both intrinsic and extrinsic properties of tumorigenesis, including angiogenesis and immune evasion, it is important to also recognize that a role for IL-1β has been described in promoting anti-tumor immunity in specific contexts. This includes a report describing the delivery of systemic IL-1β distant from the tumor site to effectively condition adoptively transferred T cell populations to generate improved anti-tumor immune responses in a B16 melanoma model [41]. Additional groups utilizing syngeneic murine tumor model systems have reported on the anti-tumor properties of IL-1β [42][43]. Indeed, IL-1β signaling in dendritic cells has been shown to be critical for the induction of radiation-induced anti-tumor immune responses [44]. The exact underlying reason for this seemingly discrepant data is generally believed to be associated with the context of IL-1β signaling, while the local concentration and secretion kinetics of IL-1β may also influence downstream outcomes following the activation of this pathway. These disparate results further emphasize the importance of validating pre-clinical data with correlative studies in cancer patients.

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