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Review Article

Nrf2 Related Immune Response to Tumor Development in Mice

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Abstract

NRF2 has gained great attention in recent years due to its role in tumor initiation and development. NRF2 is a transcription factor that regulates more than 500 genes to protect cells from oxidative damage. Nrf2 was shown to protect against tumor initiation; however, its activation at the intermediate and the late stage of tumors has been found to support tumor growth and metastasis. This dual role depends on its activation in different cells at different stages of tumor development. This mini review discusses the activation of Nrf2 in normal, tumor and immune cells during tumor initiation and development. The focus of this mini review is on the interaction between immune cells and cancer cells through Nrf2 activation and how this might contribute to tumor initiation and metastasis. This review brings attention to several important pathways that should be considered when using NRF2 as a therapeutic target in cancer.

Keywords: *Nrf2; Immune Response; Tumor Development; Mice*

The role of Nrf2 in cancer immunosurveillance and immunoediting

The immune system is made up of cells and organs that are programmed to work together to defend the body from invaders and foreign substances. For example, lymphocytes survey the tissue and organs for nascently transformed cells to protect the long-lived organisms from neoplastic diseases by a mechanism called "cancer immunosurveillance" (Figure 1a) [8]. The hypothesis of cancer immunosurveillance presents the interaction between the immune cells and cancer cells (Figure 1b); however, the immune system may not always be able to recognize the primary tumors with reduced immunogenicity [33]. As a result, tumor cells can escape the immune recognition and destruction and edit the immune cells within the tumor in a way to support tumor development; this process called "cancer immunoediting" [11]. Three phases are identified in cancer immunoediting: elimination (cancer immunosurveillance), where the abnormal cells can be eliminated by immune cells such as natural killer cells (NK); T and B-cells and by expression of some cytokines such as type I and II interferons (IFNs) and perforin in an innate immune response [11,12]. However, the elimination of the transformed cells by immune system could lead to a reduction in the immunogenicity of tumor microenvironment, where tumor cells start to adapt to stress in a hostile microenvironment that characterized by the escape of immune cells, the balance between proliferation and apoptosis, cell cycle arrest, non-angiogenic feature and chemotherapy resistance (equilibrium phase) [14]. Eventually, when the tumor size increases, the tumor cells start to adapt several pathways where several mechanisms could aid the tumor to escape from immune attack (cancer progression and metastasis) (Figure 1c and 1d).

NRF2 is a transcription factor that is activated in response to oxidative stress; it interacts with small Maf proteins and binds to the promoters of cytoprotective and antioxidative genes to induce their regulation to protect against many diseases driven by unresolved inflammation including cancer. Indeed, NRF2-mediated antioxidant response represents one of the major defense mechanisms that facilitate cell survival under any toxic effect. Severe immune dysregulation was observed as early as 30 minutes in lungs of Nrf2-deficient mice that was indicated by the expression of large numbers of proinflammatory genes related to the innate immune response [39]. This indicates that Nrf2 plays an important role in the innate immune response. Studies have shown that the activation of Nrf2 has a role in expression of cytokines and chemokines (e.g. IL-17D and CCL2) that lead to natural killer (NK) cells and monocytes infiltration into tumors (Figure 1a and 1b) and that IL-17D$^{-/-}$ mice showed higher incidence of tumors compared to WT-mice [23,28,30]. Additionally, the proinflammatory cytokine, IL-17, the Nrf2-T helper cell (Th)17 axis, found to confer protection against tumorigenesis and viral infection [30]. Moreover, a recent study showed that Nrf2 plays an important role in invariant natural killer T-cell development [29], a subtype of NKT cells that produce IFNγ to activate NK, cytotoxic T-cell, and dendritic cells (DCs) to produce IL-12 [29,38] (Figure 1b).

These observations show that the expression of Nrf2 at early stage of tumor plays a vital role in eradication of the transformed cells (elimination phase) and provides a protection against carcinogenesis in normal and premalignant cells (Figure 1a and 1b). However, if the factors and the immune cells fail to completely eradicate the transformed cells, the cells will undergo an equilibrium phase where a small number of persistent tumor cells will be held in check by active immune cells [21] (Figure 1b). Previous studies have demonstrated that Nrf2 directly inhibits the transcription of cytokines related-immune cells and indirectly limit the inflammation through heme oxygenase-1 (HO-1) expression [6,20].

Nuclear factor-kB (NF-κB) complex (a family of transcription factors that includes RelA (p65), RelB, c-rel, p50 and p52) [3] is a key transcription factor that mediates immune responses to infection, inflammation and cell proliferation by infiltration of many immune cells such as lymphocytes, macrophages, monocytes and plasma cells as well as the production of inflammatory cytokines [34]. Proinflammatory cytokines such as tumor necrosis factor TNFα, interleukin IL-1β and lipopolysaccharide (LPS) are among the most potent NF-κB activators [3,31,34]. However, the direct infiltration of many immune cells and the production of inflammatory cytokines lead to chronic inflammation that could develop to carcinogenesis [19]. Nrf2 was found to modulate the NF-kB activation; and that high NF-kB activation was demonstrated in Nrf2$^{-/-}$ mice compared to wild type mice following lipopolysaccharide (LPS) administration [39]. Moreover, Nrf2 chromatin immunoprecipitation (ChIP)-seq analyses revealed that Nrf2 binds to the proximity of the proinflammatory cytokine genes, including IL6 and IL-1β and inhibits LPS-induced expression of these genes [20]. Taken together, these findings show that Nrf2 activation can attenuate pro-inflammatory stimuli that lead to decrease inflammation and inflammatory damage in normal cells and suppress carcinogenesis especially in its earliest stages; this topic has been extensively reviewed previously [16-18]. However, this may suppress the immune response in cancer and support the repair and the growth of tumor cells, suggesting the protective role of Nrf2 in tumor and tumor immunoediting.

Furthermore, Nrf2 found to be involved in mechanisms to suppress the proinflammatory Th1 and Th17 responses and contribute to the activation of T-regulatory and Th2 cells; in addition to its role in the differentiation of DCs and macrophages [9]. Loss of Nrf2 in DCs was shown to alter the function of DCs by increasing the costimulatory molecules (MHCII and CD86) and so activates T-cells [2], implicating DCs Nrf2 activation in T-cell activity inhibition and tumor progression (Figure 1b).

In cancer, NK cells found to be conditioned to lose their cytotoxicity function and gain cytokine production phenotype [25]. Recent study showed that the activation of T-cell Nrf2 by tBHQ decreased murine NK cell percentage, IFNγ, granzyme B and perforin and induction of cell surface proteins (CD25 and CD69)/markers of NK cell activation [7]. Although, NK cells and M1-macrophages recruitment increase in response to higher IL-17D through the activation of Nrf2 in tumor cells, lower IL-17D in certain high grade and metastatic tumor was observed which might provide a favorable microenvironment for tumor growth by inhibiting the immune cells infiltration into the tumor [28,30].

Based on studies reviewed; in mice, Nrf2 has a protective role against tumorigenesis in earlier stages of tumor development. However, Nrf2 is also found to protect the tumorigenic effects during metastasis; however, this depends on whether the activation of Nrf2 occurs in tumor or in immune cells. But in all cases, the protection effects of Nrf2 occur through mechanisms by which the antioxidants and cytoprotective genes are increased, and so cell death (by apoptosis and necrosis) is decreased which ultimately produce a more favorable microenvironment for tumors.

Cancer immunoediting related to aberrant Nrf2 activation

Tumor microenvironment is composed of a heterogenous mixture of cells, factors, cytokines and immunocytes. The expression of Nrf2 in tumor cells was found to occur through several pathways such as somatic mutations in Keap1; epigenetic silencing of Keap1 expression; accumulation of disruptor proteins such as p62; transcriptional induction of Nrf2 by oncogenic K-Ras, B-Raf and c-Myc; and post-translational modification of Keap1 cysteines [1,10,24,35,37,42]. The commonly high Nrf2 activity in cancer may play a role in the formation of this complex mixture. Studies showed that the cross talk of Nrf2 activated by immune cells and Nrf2-tumor cells may contribute to tumor angiogenesis and invasion via production of cytokines [22,40]. A recent study showed that tumor cells could activate Nrf2 in macrophages through the production of lactate; where high CD163 and Arg1 and low IL-1b and IL-6 skew the differentiation of macrophages towards M2-macrophages [13]; in turns, M2-macrophages high Nrf2 expression promotes the vascular endothelial growth factor (VEGF) to support the angiogenesis [13] (Figure 1c and 1d). Actually, chromatin immunoprecipitation (CHIP-seq and CIP-qPCR) analyses revealed that Nrf2 binds to the proximity of IL-1b and IL-6 in macrophages and inhibit RNA Pol II recruitment [20]. Furthermore; the activation of T-cell Nrf2 in mice showed high frequency of intrarenal CD25+ FOXP3+ T-reg cells which was accompanied with decreased frequencies of CD11b+ CD11c+ dendritic cells and F4/80 macrophages and low levels of TNF-□, IFNɣ and IL-17 [27] (Figure 2d). However, a study showed that the systemic activation of Nrf2 in Scurfy (Sf) mice was found to suppress the effector T-cell activities independently of T-reg cells [36].

Among several immune cells that are attracted to the tumor, the myeloid-derived suppressor cells (MDSCs; their phenotype in mice is $CD11b^{+}Gr1^{+}$), the heterogenous population of cells that include myeloid progenitors and immature macrophages, immature granulocytes and immature dendritic cells, found to have a vital role in immune cells suppression in tumor [15]. In addition to their non-immunological function that represented by the promotion of tumor, MDSCs support the angiogenesis and tumor-cell invasion and metastasis [26]. MDSCs of Nrf2-deficient mice was found to retain high levels of ROS compared to WT-mice; and using $Nrf2^{+/+}$ and $Nrf2^{-/-}$ BALB/C and C57BL/6 mice bearing 4T1 mammary carcinoma and MC38 colon carcinoma, Nrf2 shown to increase the infiltration of MDSCs and enhance MDSC suppressive activity in cancer through ROS regulation, where high ROS-MDSCs attack the $CD8^{+}$ and inhibit their activity through T-Cell Receptor (TCR) which found to supports the metastasis (Figure 1d) [5,32]. These observations confirm the immune suppressive function of Nrf2 in cancer. However, recent study showed that WT-Nrf2 in mice might provide a protection in non-small cell lung cancer (NSCLC) by expressing a "favorable" immune cells and cytokines compared to KO-Nrf2 mice [41].

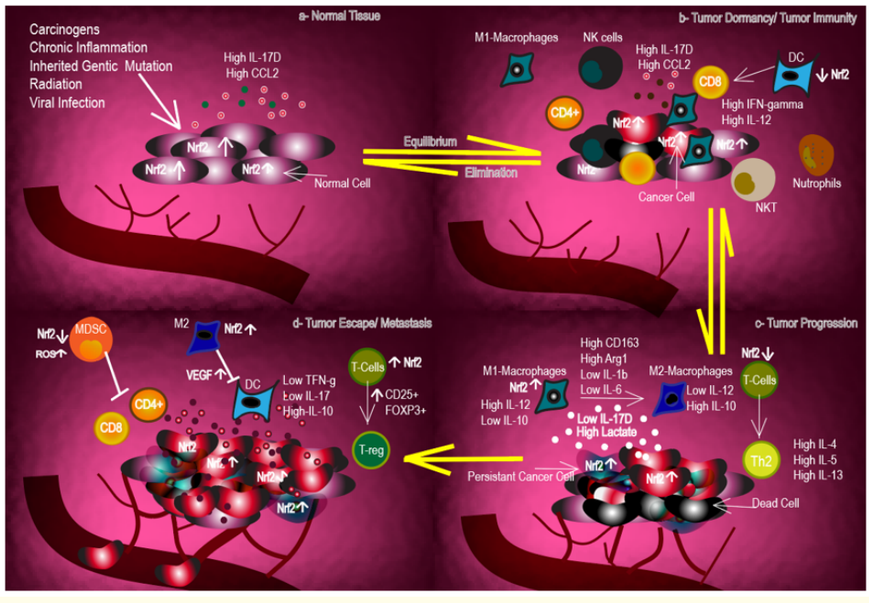

Figure 1: *The role of Nrf2 in immunosurveillance and immunoediting. Normal cells activate Nrf2 in response to oxidative stress to express cytokines (a) which contribute to the recruitment of different immune cells (e.g. Natural Killer cells NK; Invariant Natural Killer T-cells (I.NKT), Dendritic Cells (DC), Macrophages and Lymphocytes) where tumor cells become in equilibrium with immune cells (b); the activation of Nrf2 in immune cells versus the tumor cells-Nrf2 activation contributes to the differentiation of immune cells towards lower immunogenicity which favors the growth of tumor (c); the higher and/or lower Nrf2 activity in tumor cells and the newly differentiated immune cells support the invasion and the metastasis of tumor (d).*

The activation of Nrf2 in normal cells might give different responses compared to Nrf2 activation in cancer cells, which was found to be different from Nrf2 activation in different immune cells. It is also important to notice that the cross talk between Nrf2 of tumor cells and immune cells Nrf2 could contribute to the immune response to tumor development which could support tumor progression and invasion. Additionally, Nrf2 deficiency in some immune cells (e.g. MDSCs) might support the tumor growth and metastasis.

Conclusion

These observations tell us that it is essential to understand the Nrf2 activation in immune cells and cancer cells in different stages of cancer. Therapies that are used to activating or suppressing Nrf2 need to be further studied to better understand how these therapies control the activation of Nrf2 in normal, tumor and immune cells; and how this increases the therapeutic usefulness of Nrf2 inhibitors or activators in patients with primary or invasive cancer.

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Volume 9 Issue 2 February 2021