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Engineering Grp170-based immune modulators for cancer immunotherapy

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We have recently demonstrated that glucose-regulated protein 170 (Grp170), a stress-responsive molecular chaperone of the endoplasmic reticulum, can be exploited to stimulate anticancer immunity due to its superior antigen chaperoning and delivering capacity. The immune remodeling of the tumor microenvironment induced by a Grp170-based chaperone leads to immune responses that effectively control the progression of both primary neoplasms and their metastases. Our findings support the development of Grp170-based immunomodulating strategies to potentiate antitumor immune responses.

Most, if not all, heat shock proteins (HSPs) are constitutively expressed by living organisms and function as molecular chaperones to maintain protein homeostasis. Among these abundant and evolutionarily conserved molecules, high molecular weight chaperones such as glucose-regulated protein 170 (Grp170, also known as oxygen-regulated protein 150, ORP150) are distantly related to the HSP70 family, yet have received much less attention.[1] The most distinctive feature of these large chaperones is their superior capacity to hold client proteins or denatured substrates.[2,3] Cancer cells often express high levels of stress-responsive proteins including HSPs, possibly owing to an increased need for chaperoning functions ensuing the intense synthesis of mutated and misfolded proteins. Thus, stress-responsive proteins (e.g., Grp170) isolated from malignant cells are expected to carry tumor-associated antigens (TAAs) and may offer a personalized, polyvalent vaccine therapy.[4,5] Recombinant vaccines generated by complexing Grp170 with known TAAs (e.g., gp100) have been shown to effectively promote a tumor-specific cytotoxic T-lymphocyte (CTL) response.[3,6] A Phase I clinical trial testing a similar approach is currently underway. Our studies reveal that the chaperoning properties of Grp170 are required for its ability to modulate immune functions (e.g., to interact with antigen-presenting cells, promoting antigen shuttling and cross-presentation).[6]

The deletion of the endoplasmic reticulum (ER) retention sequence of Grp170 results in its secretion into the extracellular environment. Interestingly, the enforced expression of such a secretable Grp170 variant by several poorly immunogenic cancers markedly reduces tumor growth in vivo but not in vitro.[2] Extracellular Grp170 appears indeed to act as an activation signal for dendritic cells (DCs) and as a shuttle for the delivery of TAAs to antigen-presenting cells.[2] Natural killer (NK) cells and CTLs play a major role in the establishment of protective, long-term antitumor immune response. In spite of the fact that cancer cells contain a large repertoire of (often undefined) TAAs, simply killing malignant cells with conventional chemotherapeutics may be insufficient to achieve effective tumor-targeting immune responses. In line with its ability to facilitate antigen cross-presentation, the direct intratumoral delivery of Grp170 via a non-replicating adenovirus has been shown to promote the immunogenic demise of tumor cells, thereby improving the effectiveness of a targeted anticancer agent.[7]

The immunosuppressive nature of the tumor microenvironment (TME) constitutes a major hurdle against successful cancer immunotherapy, through a number of mechanisms. Based on the fact that engaging innate pathogen-sensing signaling pathways enhances antigen recognition and hence adaptive immune responses, we have recently constructed a chimeric chaperone by strategically incorporating a pathogen-derived, NFκB-stimulating signal (i.e., flagellin) into Grp170.[8] We hypothesized that coupling TAAs and a danger signal into the same chaperone-based delivery system would ensure optimal T-cell priming by DCs.[9,10] We named this multifunctional molecule Flagrp170, which turned out to have the dual ability to facilitate the cross-presentation of TAAs while inducing the functional activation of DCs.

The intratumoral delivery of an adenovirus encoding Flagrp170 provokes a highly effective response that targets both primary lesions and distant metastases. This response is much more robust than

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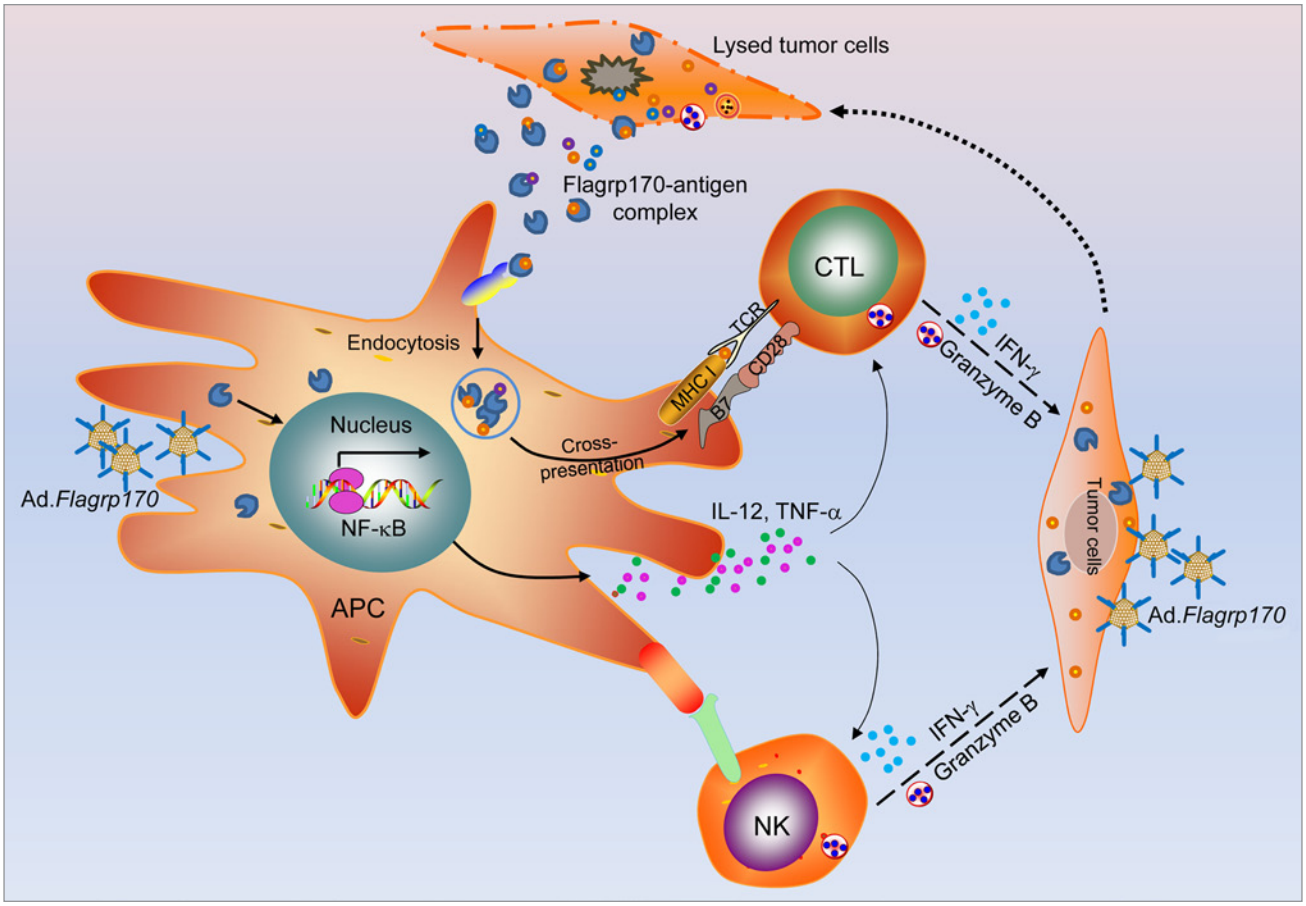

Figure 1. The immunomodulating effects of a multifunctional chimeric chaperone in the tumor microenvironment. Flagrp170 produced by tumor cells delivers tumor-associated antigens to antigen-presenting cells (e.g., DCs) for efficient cross-presentation and T-cell priming. The NFκB signaling pathway is triggered in DCs upon infection with Flagrp170-encoding adenoviruses as well as by extracellular Flagrp170, resulting in their functional activation. The upregulation of co-stimulatory molecules and the production of inflammatory cytokines (e.g., interleukin-12, IL-12) promote the effector functions of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs). These cells, possibly via interferon γ (IFNγ) and granzyme B, kill malignant cells, hence causing the release of Flagrp170-tumor antigen complexes that may further amplify T-cell antitumor responses.

that elicited by the administration of flagellin or unmodified Grp170 and has been documented in murine models of various tumors, including melanoma, prostate cancer and colon carcinoma.[8]

The intratumoral delivery of Flagrp170 mobilizes a systemic, polyvalent tumor-reactive CTL response that targets several natural TAAs. Intratumoral Flagrp170 preferentially polarizes the TME toward a T_H1/T_C1 response, as evidenced by marked elevations in the local levels of interleukin-12 (IL-12) and interferon γ (IFNγ).[8] These two cytokines turned out to be required for Flagrp170-induced anticancer immune responses, as their neutralization abolished the therapeutic efficacy of Flagrp170. Increased levels of IFNγ in the TME can be attributed to tumor-infiltrating NK and T cells, especially $CD8^+$ T cells. In line with notion, the antitumor effects of Flagrp170 depend on NK and $CD8^+$ T but not $CD4^+$, T cells. In addition, $CD8\alpha^+$ DCs are required for the antineoplastic activity of Flagrp170, presumably owing to their major role in cross-priming, as defects in the $CD8\alpha^+$ DC compartment significantly reduced the frequency of IFNγ-expressing $CD8^+$ T cells infiltrating neoplastic lesions in response to Flagrp170 as well as its general antitumor effects. Surprisingly, DCs do not secrete high levels of IL-12 in the microenvironment of Flagrp170-treated tumors, suggesting that other unknown cell types may also contribute to Flagrp170-induced immune activation.

Our studies provide some insights into the therapeutic effects of Flagrp170. However, further investigation is needed to elucidate the precise molecular and cellular mechanisms that underlie the TME-remodeling activity of Flagrp170 (**Fig. 1**). Although chronic inflammation has been linked to tumor progression, our findings suggest that appropriately altering the inflammatory properties of the TME may significantly potentiate antitumor immune responses. As Flagrp170 selectively stimulates the NFκB signaling pathway in DCs but not in tumor cells, the therapeutic manipulation of inflammatory responses in the immune compartment of tumors may be essential to break immune tolerance.

In summary, engineered immune chaperones may represent highly effective, non-toxic immunomodulating agents that can be exploited to "condition" the immunosuppressive/tolerogenic TME to

maximize the efficiency of anticancer vaccines. Additional studies are warranted to assess the therapeutic potential of these immune modulators combined with conventional chemotherapeutic for the treatment of metastatic malignancies.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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