Innate Cancer Immunoediting

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Immune cells detect and destroy cancer cells; however, very early changes in cancer genome and phenotype coupled with immune system selection cause escape variant survival in a process called cancer immunoediting. Although adaptive immunity is important for this process, the report by Kubick et al. provides novel insights into the role of innate immune cells for immunoediting of early transformed epithelial cells.

INTRODUCTION

Cancer immunoediting is the process where immune system components protect the host against tumor development and/or enhance tumor escape either by sculpting tumor immunoregularity or attenuating antitumor immune responses (Schreiber et al., 2011). This process includes three self-explanatory phases: elimination, equilibrium, and escape. Although the cancer immunoediting process serves as a foundational platform for cancer immunotherapy (O’Donnell et al., 2019), very few studies have captured cancer immunoediting in its earliest stages. Now, Kubick et al. (2019) have developed an elegant fluorescent tracing mouse skin transplantation model to study the role of immunoediting in somatic epithelial cancer development. Using this system, Kubick et al. observe that innate immune deficiency permits rapid tumorogenesis, whereas early transformed clones undergo elimination or equilibrium in immunocompetent mice (Kubick et al., 2019). The established equilibrium occurs in the hair follicles that were previously thought to be immune-privileged sites. This elegant study presents a novel experimental model in which to validate the principles of cancer immunoediting previously established in sarcoma models and to explore innate immune control of early transformation.

Models of cancer immunoediting

Modeling and tracking immunoediting in a manner that faithfully recapitulates human cancer initiation remains a major goal of cancer immunoediting research. Cancer elimination is difficult to observe and is inferred in most studies by earlier onset and increased incidence of cancer in immunocompromised mice compared with immunocompetent control mice. Subcutaneous inoculations of the carcinogen 3-methylcholanthrene induces fibrosarcoma formation and have been used to study the three phases of cancer immunoediting, including the equilibrium phase and neoantigens in tumors that provoke T cell–mediated immunity (Koebel et al., 2007; Matsushita et al., 2012; Shankaran et al., 2001; Smyth et al., 2000). However, this model has not been easily tractable at its earliest phases, where it is inferred from cell depletion experiments and various

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Clinical Implications

- Tumors originating from keratinocytes undergo immune surveillance, editing, and escape.
- Innate immune cells contribute to the immunoediting process in the skin.
- The hair follicle provides a niche for immune evasion by epithelial cancer.

gene-engineered mouse strains that natural killer (NK) cells play a major role in preventing sarcoma initiation (Gao et al., 2017; Smyth et al., 2000). In the same model, O’Sullivan et al. found that innate immune cells (NK cells, M1 macrophages, and IFN-γ) could manifest cancer immunoediting activity in the absence of adaptive immunity (O’Sullivan et al., 2012). Epidermis-specific upregulation of Rae-1 stress ligand was also shown to induce rapid, coincident, and reversible changes in the organization of tissue-resident Vγ5Vδ1 TCRγδ+ intraepithelial T cells via their cytotoxic lymphocyte activating receptor NKG2D, where local Vγ5Vδ1+ T cells limited carcinogenesis (Strid et al., 2008). But immunoediting was not evaluated in this context.

Kubick et al. set out to trace somatic carcinogenesis using specific driver events (Cre recombination of KrasLSL-G12D and p53Δfl/fl alleles) in isolated cells surrounded by an initially unaffected microenvironment (Kubick et al., 2019). To avoid artificial tolerance in the autochthonous model, a skin transplantation method was employed, and this transplantable and inducible squamous cell carcinoma (SCC) model allowed intravital tracing of keratinocyte-derived cancers in different immune-deficient mouse strains. The authors favored the transplant model because they believed that it best represents the somatic-only transformation that occurs in human tumors compared with autochthonous models, and autochthonous models may misrepresent the T-cell repertoire by generating artificial tolerance to conditional alleles. Tighter inducible systems may be required for studying cancer immunoediting to obviate the need to transplant somatic cells with transformation potential, but this has been a technical challenge. At the other end of the spectrum of modeling, Park et al. (2019) and Takeda et al. (2017) described the editing of model antigens from transplanted mouse tumor cell lines by tissue-resident memory T cells and IFN-γ as critical. These models are valuable, but not the same as cancers developing de novo in the presence of an immune system.

Elaborate time-course imaging by Kubick et al. of the transformed clones was possible, and their ability to give rise to tumors in Rag1γc-deficient mice was striking (Kubick et al., 2019). Clones transitioned from hyperproliferative neoplasia to papillomas, then to larger ulcerated SCCs. Tumor latency was longer in Rag1-deficient mice and even longer in fully immunocompetent wild-type (WT) mice. Full elimination was only observed in WT mice. The authors did find that minimal transformation consistently generated a robust immunoediting response. Elimination (EGFP+ clonal loss) and equilibrium (tumor-free maintenance of EGFP+ clones) was detected by longitudinal confocal imaging, but the authors arbitrarily used timeframes as cut offs for the equilibrium and escape phases, rather than any particular molecular definition. Such signatures can now be pursued in this novel model.

Innate cancer immunoediting

The maintenance of immunoediting in Rag1-deficient mice was intriguing, suggesting that NK cells or other innate lymphoid cells (e.g., ILC1) may do the heavy lifting of early immunoediting that precedes the antigenicity required for a subsequent T-cell response. The author’s findings for a minimal role of T cells in controlling transformed epithelial cells is a clear deviation from the fundamental aspect of the current immunoediting paradigm (Kubick et al., 2019). The inability to detect NK cells in skin during the dynamic process of immunoediting as described by Kubick et al. dampens the strength of the conclusions regarding NK cells being the dominant mediators of immunoediting. The authors were only able to detect rare NK cells in the skin graft regions, and this analysis likely includes tissue-resident ILC1. In fully immunocompetent WT mice, Kubick et al. observed F4/80 macrophages proximal to the early transformed clones and some of these showed intracellular EGFP signals, suggesting phagocytosis of transformed clones. Such cells might require γc chain cytokines for their function, if not for their development, or the crosstalk with innate γc chain—dependent NK cells and other γc chain—dependent innate T cells may be necessary for effective elimination. This would be consistent with the findings of O’Sullivan et al. (2012), but here in epithelial rather than mesenchymal transformation.

EGFP+ lesions that persisted beyond the initial elimination phase exhibited or contained circular morphology, indicative of a hair follicle opening at the skin surface. Transformed lesions resided in or originated from hair follicles (Kubick et al., 2019). Retrospective analysis of eliminated clones revealed a lack of hair follicle involvement, suggesting that intrafollicular clones are relatively susceptible to elimination (Figure 1). The immunoprivileged state of hair follicles is mainly defined in association with T-cell immunity. It is not well understood how innate immune cells may be regulated in the hair follicle. Although these data demonstrate that in the absence of T and B cells, innate γc chain—dependent immune cells can mediate immune equilibrium, they do not exclude a role for T cells in immunoediting in an intact immune system, because functionally Rag1-deficient mice displayed no elimination and more escape compared with fully immunocompetent mice. It is highly likely that there is a continuum of immunoediting, both innate and adaptive, when elimination fails. Further studies will be required to understand the mechanisms driving this innate immunoediting of early transformation. Importantly, we need to understand and identify the key immune cells that are required for innate editing of early transformation. The complexity of the model, although necessary for creating the most physiologically relevant conditions possible, limits the amount of material for phenotyping. This explains the paucity of cancer
Immunoeediting studies in general. Cell depletion studies in WT mice to replicate the findings in Rag1γc-deficient mice will also be important. Although neoantigens have been clearly illustrated to be targets of T-cell cancer immunoeediting (Matsushita et al., 2012), the neoantigen loads at the onset of transformation are poorly understood. In this regard, whole genome sequencing of the lesions from WT, Rag1−/−, and Rag1γc−/− mice would be of great interest. Any pathways and/or molecules involved in innate immunoeediting remain to be determined. These may be quite an assortment based on the broad repertoire of innate immune mechanisms, with stress-induced NKG2D-NKG2D ligands being one example (Strid et al., 2008), but likely macrophage-dependent mechanisms may be revealed. In any case, Kubick et al. (2019) see evidence of immunogenicity and editing with a minimal level of transformation in transformed epithelial cells.

The elegant model presented by Kubick et al. (2019) could serve as a novel screening platform for early preventative therapies targeted against immune-controlled cancers. Discovery of the key cells and molecular pathways using this kind of system may open up the possibility of preventative approaches to limit cancer development. This would be particularly applicable to cancers originating in mucosal surfaces (skin, gastrointestinal tract, and lung) where a high rate of mutation because of environmental stresses may put the protective innate immune system under duress.

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**CONFLICT OF INTEREST**
MJ has research agreements with Bristol Myers Squibb and Tizona Therapeutics and is on the scientific advisory board of Tizona Therapeutics and Compass Therapeutics. TB has research agreements with ENA Therapeutics and is on the scientific advisory board of Oncomyx.

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**REFERENCES**

**Figure 1. Innate cancer immunoeediting of transformed epithelial cells in the hair follicle.** Although immune cells can effectively eliminate transformed epithelial cells in the intralocular skin, innate immune cells are responsible for immunoeediting in the hair follicle. ILC, innate lymphoid cell; NK, natural killer.