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Complement C5a-Mediated TAM-ing of Antitumor Immunity Drives Squamous Carcinogenesis

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https://doi.org/10.1016/j.ccell.2018.09.005

In this issue of *Cancer Cell*, Medler et al. demonstrate that fibrinolytic enzyme-mediated generation of complement C5a reprograms tumor-infiltrating C5aR1$^+$ macrophages into an immunosuppressive phenotype that dampens CD8$^+$ T cell responses during squamous carcinogenesis. C5aR1 blockade combined with chemotherapy offers a promising immunomodulatory strategy for treating squamous cell carcinoma.

Identifying molecular cues that guide immune responses in the tumor microenvironment (TME) remains a challenge for immuno-oncologists and a focal point for overcoming immunosuppressive barriers and designing effective immunotherapies. It is well accepted that neoplastic progression can be modulated by signals originating from both innate and adaptive immune compartments that converge in the TME (Mantovani et al., 2008). Complement, an evolutionarily conserved branch of innate immunity, is known for its ability to rapidly respond to pathogens and foreign material through an elaborate network of pattern recognition molecules, cell-activating receptors, and proinflammatory effectors (Hajishengallis et al., 2017).

Traditionally, the perception of complement in tumor immunology was that of an ancillary innate immune system that potentiates the cytolytic action of anti-tumor antibodies through deployment of complement receptor- and FcγR-dependent phagocytic pathways (Reis et al., 2018). Marking a paradigm shift in tumor immunology, growing evidence over the last decade has revealed broadly protumorigenic functions of complement in the TME. Complement C3 activation and downstream C5a-C5aR1 signaling were first shown to foster tumorigenesis by modulating immunosuppressive pathways in a model of cervical cancer (Markiewski et al., 2008). Ever since, a surge of studies has substantiated the pivotal role of complement-driven inflammation in tumorigenesis (Reis et al., 2018). C5a, a proinflammatory peptide generated by both canonical (complement-dependent) and extrinsic routes (coagulation/fibrinolytic proteases) (Huber-Lang et al., 2006; Hajishengallis et al., 2017), has been implicated in cancer cell proliferation, myeloid suppressor cell function, inflammatory cytokine secretion, pro-angiogenic signaling, and tumor metastasis (Reis et al., 2018).

The K14-HPV16 model of *de novo* epithelial carcinogenesis drives skin malignant transformation by targeted HPV16 transgene expression in basal keratinocytes (Andreu et al., 2010). Previous studies have indicated that, whereas HPV16 mice develop skin neoplasias in a B cell-, antibody- and FcγR-dependent manner, complement C3 activation appears dispensable in this process (de Visser et al., 2005; Andreu et al., 2010; de Visser et al., 2004). To date, the precise mechanistic involvement of complement effectors in squamous neoplastic progression has remained ill-defined. In this issue of *Cancer Cell*, Medler et al. provide evidence for a C3-independent, tumor-promoting role of complement C5a during squamous carcinogenesis (Medler et al., 2018).

First, the authors confirm that neoplastic progression in C3-deficient HPV16 mice involves chronic inflammation mediated by C5a. This notion is supported by pronounced C5a accumulation in dermal stromal regions in close proximity to premalignant dysplasias in these mice. Investigating the consequences of C5a release, the authors reveal prominent infiltration of tumors by C5aR1$^+$CD45$^+$ leukocytes, including mast cells, macrophages, CD11c$^+$ dendritic cells, and Gr1$^+$ granulocytes. The authors then provide robust evidence for a protumorigenic role of C5aR1 signaling in their model. Consistently, the growth of orthotropic squamous cell carcinomas (SCCs) was significantly retarded in recipients lacking C5aR1.

In eloquently designed myeloid cell/tumor cell co-engraftment experiments in C5aR1$^{-/-}$ recipients, they demonstrate that the protumorigenic effect of C5a in K14-HPV16 mice is mainly mediated by C5aR1$^+$ mast cells and macrophages. They further substantiate a prerequisite role for C5aR1 by observing significantly reduced CD45$^+$ leukocyte infiltration, attenuated keratinocyte proliferation, and lower SCC incidence in HPV16/C5aR1$^{-/-}$ mice. Collectively, these findings support the notion that locally generated C5a acts on C5aR1$^+$ mast cells and macrophages, which in turn propagate an immunosuppressive milieu fostering squamous carcinogenesis.

Corroborating that upstream C3 activation is dispensable for C5a generation in this tumor model, the authors show that C5a is generated by a C3-bypass mechanism involving fibrinolytic enzymes enriched in SCCs, such as urokinase plasminogen activator (uPA) and plasmin. Plasmin-generated C5a targets infiltrating C5aR1$^+$ myeloid cells and skews the TME toward an M2 phenotype that dampens CD8$^+$ T cell responses.

Of note, a strong correlation between M2-macrophage polarization and uPA

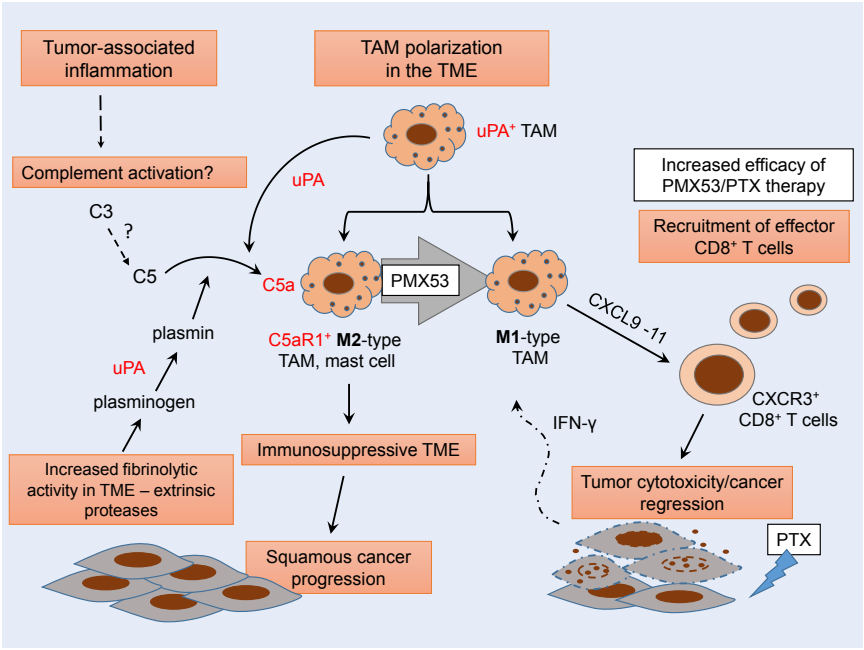

Figure 1. A Schematic Outline of the Proposed Mechanism by which the C5a-C5aR1 Axis Becomes Operative in the TME and Impacts Key Immunomodulatory Processes that Drive Neoplastic Progression in the K14-HPV16 Skin Cancer Model

Medler et al. (2018) demonstrate a convergent role of fibrinolytic pathways and complement C5a in reshaping the TME by skewing TAMs toward an M2-like phenotype that sustains immunosuppression. The increased efficacy of combined C5aR1 blockade (PMX53 treatment) and chemotherapy (paclitaxel, PTX) in this model pave the way to likely more effective immunomodulatory treatments for skin cancer.

upregulation is revealed, indicating that M2-type protumorigenic macrophages may selectively favor the generation of C5a in SCCs via uPA-dependent proteolytic activation (Huber-Lang et al., 2006). uPA$^+$ M2-polarized macrophages mediated prominent C5a generation *in vitro* in the presence of serum and uPA$^+$ SCC cells. Interestingly, C5a generation in this setup appeared to involve both plasminogen- and C3-dependent routes, indicating that C3 may partly contribute to the accumulation of C5a in the TME.

Medler et al. (2018) showed that lack of uPA activity *in vivo* correlates with decreased C5a generation in the dermal stroma and attenuated infiltration of C5aR1$^+$ CD45$^+$ leukocytes into HPV16 tumors. While these findings argue for a cardinal role of fibrinolytic pathways in generating C5a in SCCs, further studies are warranted to rule out the contribution of C3 activation in this process. In fact, these findings allow room for speculating that a C3-mediated effect in the HPV16 model may be partially masked by the fibrinolytic activity in these tumors. We should emphasize that, while transgenic models are valuable for modeling disease pathophysiology, they often elicit extreme phenotypes with broader impact on a systems level and do not always recapitulate the relative contribution of distinct pathogenic drivers in the clinical landscape. For instance, genetic C3 deficiency has been associated with an overactive fibrinolytic system and higher plasma levels of thrombin (Huber-Lang et al., 2006). This presumably compensatory adaptation in C3$^{-/-}$ mice might have important implications when interrogating the role of C3 in the HPV16 cancer model.

Adding a translational component to their work, the authors showed that C5aR1$^+$ leukocyte infiltration is a histological hallmark of human squamous carcinogenesis, with large numbers of C5aR1$^+$ cells being detected in human SCC biopsies. Retrospective analysis of a large dataset of human SCCs revealed a significant correlation between longer survival of patients and low intratumoral C5aR1 expression.

Furnishing a therapeutic perspective, the authors show that C5aR1 antagonism (PMX53 treatment) synergizes with chemotherapy (paclitaxel, PTX) in restricting the growth of orthotopic SCC implants. Of note, this finding reverberates in a different tumor setting the efficacy of combining C5aR1 modulation with established anti-cancer regimens that reinstate anti-tumor T cell responses (Markiewski et al., 2008; Reis et al., 2018). The profound impact of C5aR1 blockade on both the TME and lymphoid compartment is underscored by the transcriptional reprogramming of C5aR1$^+$ tumor-associated macrophages (TAMs), enhanced recruitment of CXCR3$^+$ CD8$^+$ effector T cells, and peripheral priming of antigen-dependent anti-cancer T cell responses. Finally, a functional impact of C5aR1 signaling on anti-tumor T cell responses was corroborated *in vivo* by pronounced infiltration of both effector and memory subsets of CXCR3$^+$ CD8$^+$ T cells into implanted SCCs and by a prominent cytotoxic signature in tumors from PTX/PMX-53-treated mice.

In their study, Medler et al. address fundamental questions related to how complement effector pathways modulate tumorigenic responses in the backdrop of chronic inflammation (Figure 1). They provide insight into mechanisms by which C5a modulates anti-tumor immunity in squamous carcinogenesis. More importantly, the C5a-C5aR1 axis is identified as a tractable target for developing combinatorial immunotherapies for squamous carcinomas. In contrast to recent studies indicating a pro-tumorigenic role of C3 in carcinogen-induced models of skin cancer (Bonavita et al., 2015), the present study argues for a dispensable role of C3 in viral transgene-driven squamous carcinogenesis. The distinct nature of these tumor models illustrates a contextual involvement of complement effectors in skin carcinogenesis, depending on the triggering mechanism. Medler et al. hypothesize that the heightened complement regulatory arsenal of SCCs may thwart canonical C3 activation, thus favoring C5a generation by fibrinolytic proteases that are highly enriched in neoplastic tissues. While this plausible mechanism remains to be validated in different tumor models, Medler et al. provide a basis for better understanding the diverse triggers of complement activation in the TME and how these likely dictate the contextual and hierarchical involvement of complement-dependent effector pathways in cancer development.

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DECLARATION OF INTERESTS

J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors for the treatment of complement-mediated inflammatory disorders (including third-generation compstatin analogs such as AMY-101), and inventor of patents or patent applications that describe the use of complement inhibitors for therapeutic purposes, some of which are developed by Amyndas Pharmaceuticals. J.D.L. is also the inventor of the compstatin technology licensed to Apellis Pharmaceuticals (4(1MeW)7W, also known as POT-4 and APL-1) and PEGylated derivatives such as APL-2. The other authors declare no competing interests.

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Intratumoral Heterogeneity: Tools to Understand and Exploit Clone Wars in AML

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https://doi.org/10.1016/j.ccell.2018.09.004

In this issue of *Cancer Cell*, de Boer et al. refine a set of acute myeloid leukemia (AML)-enriched plasma membrane markers that can be used to identify, prospectively isolate, and longitudinally track leukemic subclones within individual AML patients, correlating immunophenotypic profiles with specific mutational signatures, transcription, functional behavior, and therapeutic outcomes.

Recent advances in sequencing technologies together with reduced costs and the availability of tumor tissues have allowed for the compilation of an almost complete catalog of the mutational spectrum in acute myeloid leukemia (AML) (Papaemmanuil et al., 2016). In consonance with its clinical and biological diversity, the list of genetic alterations detected in AML is a long and heterogeneous affair. In addition, quantitative differences between variant allele frequencies (VAFs) of mutations present in individual tumors at presentation and in a small number of paired longitudinal studies demonstrating differences of mutational composition and burden at diagnosis and relapse have further revealed an equally diverse intratumoral composition, with multiple clones often co-existing in individual patients (Ding et al., 2012). Further complicating matters, it is now established that the "acute" phase of AML is preceded by a pre-leukemic process, clonal hemopoiesis, during which mutations accumulate in hematopoietic stem cells (HSCs) (Jaiswal et al., 2014). Upon full-blown transformation, the presence of specific mutations and likely combinations affect disease course and can be used as predictors of therapeutic outcomes and relapse risk.

However, despite significant advances and the promise of novel FDA-approved therapeutics, the overall prognosis of AML still remains dismal, with the great majority of patients eventually succumbing to the disease. This lack of success relates to our inability to define and isolate the leukemia stem cells (LSCs) that drive AML and generate intratumoral heterogeneity, hampering our understanding of the disease and ultimately our ability to improve outcomes. A little more than 20 years after their seminal description (Bonnet and Dick, 1997), a large number of studies have investigated LSC-specific surface markers. While in some cases, these have allowed the prospective isolation and targeting of LSCs