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Targeting tumor tolerance: A new hope for pancreatic cancer therapy?

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ABSTRACT

With a 5-year survival rate of just 8%, pancreatic cancer (PC) is projected to be the second leading cause of cancer deaths by 2030. Most PC patients are not eligible for surgery with curative intent upon diagnosis, emphasizing a need for more effective therapies. However, PC is notoriously resistant to chemoradiation regimens. As an alternative, immune modulating strategies have recently achieved success in melanoma, prompting their application to other solid tumors. For such therapeutic approaches to succeed, a state of immunologic tolerance must be reversed in the tumor microenvironment and that has been especially challenging in PC. Nonetheless, knowledge of the PC immune microenvironment has advanced considerably over the past decade, yielding new insights and perspectives to guide multimodal therapies. In this review, we catalog the historical groundwork and discuss the evolution of the cancer immunology field to its present state with a specific focus on PC. Strategies currently employing immune modulation in PC are reviewed, specifically highlighting 66 clinical trials across the United States and Europe.

1. Introduction

Pancreatic cancer (PC) is projected to be the second leading cause of cancer deaths by 2030 (Rahib et al., 2014). Systemic cytotoxic and kinase-targeted treatments represent the standard of care for most patients presenting with PC. However, the majority of tumors will intrinsically exhibit or ultimately develop resistance to these regimens and progress (Moore et al., 2007; Koay et al., 2014). As a result, both the median survival and annual death rate for patients with PC have remained unchanged over the last 20 years (Baxter et al., 2007).

As promising alternatives, therapies designed to stimulate antitumor immune responses have achieved success in patients with melanoma (Postow et al., 2015; Robert et al., 2015), non-small cell lung cancer (Brahmer et al., 2015; Rizvi et al., 2015b), mismatch-repair deficient colorectal cancer (Le et al., 2015a) and renal cell carcinoma (Motzer et al., 2015). Unfortunately, single-agent application of the most successful agents to date, specifically immune checkpoint inhibitors, demonstrated little to no effect in PC (Royal et al., 2010; Brahmer et al., 2012). These data could be interpreted as discouraging regarding the potential to harness the immune system against PC, but the data also highlight the lack of understanding of the totality of immunologic mechanisms at play within the tumor microenvironment in human PC. Multimodal immune modulation consistently demonstrates antitumor responses in mouse models of PC (Winograd et al., 2015; Soares et al., 2015a). Thus, the challenge in the field will be to translate these preclinical data into effective therapeutic combinations for patients. In this review, we discuss the background and current state of cancer immunotherapy with a specific focus on setbacks and opportunities in PC treatment.

2. Antitumor immune responses

2.1. The cancer immunoediting hypothesis

Landmark investigations in the mid-20th century demonstrated that mice can be immunized against syngeneic tumor transplants (Old & Boyse, 1964; Klein, 1966). These observations led Burnet and Thomas to propose the "cancer immunosurveillance" hypothesis. These scientists posited that the formation of small tumors may be more common than previously thought, building upon early observations by Ehrlich that the anticipated frequency of mutational events predicts an overwhelming rate of cancer (Ehrlich, 1909). The hypothesis forwarded the emergence of "new antigenic potentialities," now known as neoantigens, that leads to immune-mediated rejection before tumors are clinically evident (Burnet, 1970). However, this hypothesis underwent a setback with the development of athymic nude mice, characterized by deficient T cell development. Under the immunosurveillance hypothesis, rejection of developing tumors should be impaired and these mice should demonstrate a higher incidence of both spontaneous and carcinogen-induced tumors. However, Stutman et al. demonstrated no difference between spontaneous and MCA-induced tumor development in athymic nude mice compared to wild-type

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mice, arguing against a T cell-dependent mechanism of tumor rejection (Stutman, 1974, 1979).

The cancer immunosurveillance hypothesis was revisited a decade later, spurred by emerging evidence that athymic nude mice possess some T cell functionality (Maleckar & Sherman, 1987). Shankaran et al. evaluated MCA-induced sarcoma development in RAG2 deficient mice, characterized by disrupted V(D)J recombination and the absence of mature T and B lymphocytes (Shinkai et al., 1992; Shankaran et al., 2001). Not only did the group observe an increased incidence of MCA-induced sarcoma development in these mice, but further demonstrated that 40% of tumors generated in *rag2*$^{-/-}$ mice were subsequently rejected upon transplantation into syngeneic wild-type mice in a T cell-dependent manner (Shankaran et al., 2001). Thus, the cancer immunosurveillance hypothesis, which posits the immune system can recognize and destroy nascent transformed cells in an antigen-specific manner, regained support (Dunn et al., 2002).

Carcinogen-induced tumorigenesis in a variety of knockout mice subsequently revealed a host of immune mediators critical to functional cancer elimination during immunosurveillance. In the same line of work using *rag2*$^{-/-}$ mice, Shankaran et al. demonstrated that mice deficient in either the IFNγ receptor or its downstream mediator, stat1, developed a higher frequency of MCA-induced sarcomas, similar to that of the *rag2*$^{-/-}$ mice (Shankaran et al., 2001). Similar investigations revealed that SCID mice, also deficient in mature lymphocytes, also developed MCA-induced sarcomas at an elevated rate (Smyth et al., 2001). Essential roles in elimination during cancer immunosurveillance were subsequently demonstrated for GM-CSF (Enzler et al., 2003), perforin (Street et al., 2001, 2002), β2-microglobulin (Street et al., 2004), TRAIL (Zerafa et al., 2005), NKG2D (Smyth et al., 2005), IL12 (Langowski et al., 2006) and several other inflammatory mediators (Dunn et al., 2006).

However, the majority of clinically evident cancers have evolved to evade immunosurveillance. In order to globally address the immunologic evolution of developing tumors, the cancer immunosurveillance hypothesis was formally incorporated into a process termed "cancer immunoediting", characterized by three distinct phases: elimination, equilibrium and escape (Schreiber et al., 2011). In the elimination phase, highly immunogenic tumors are recognized and eliminated appropriately by cancer immunosurveillance mechanisms, as observed in sarcoma transplantation from immunocompromised to wild-type mice. The second phase, equilibrium, describes the immune-mediated evolution of a tumor. Equilibrium may be viewed as a balancing phase, in which the adaptive immune system successfully prevents tumor outgrowth but is unable to clear the tumor. Cancer cells may evolve during this time, developing mechanisms to reduce immunogenicity and/or promote immune tolerance. Koebel et al. elegantly described the equilibrium phase in MCA-induced sarcomas, demonstrating that depletion of T cells is sufficient to return stable tumors to a state of progressive growth and metastasis (Koebel et al., 2007). Progression to the escape phase entails the acquired capacity of tumor cells to proliferate and metastasize while avoiding immune-mediated destruction. The field of cancer immunotherapy has focused on deciphering counterregulatory mechanisms leading to immune escape, which has already contributed to considerable clinical breakthroughs with immune checkpoint inhibition. However, much has yet to be elucidated regarding the evolution of counterregulatory mechanisms and the complex interplay between them in the tumor microenvironment.

2.2. Neoantigens

We have thus far discussed a role for adaptive immunity in preventing the development of cancer, yet adaptive immunity evolves to inhibit reactivity to self-antigens. In order for the adaptive immune system to reject an autologous tumor, the tumor cells must present an antigen recognized by host T cells. These self-reactive peptides fall into one of two classes of antigens: (1) nonmutated proteins to which central tolerance was not established, such as those associated with immune privileged sites, or (2) mutated proteins not previously part of the negative selection process (Schumacher & Schreiber, 2015). For example, whole exome sequencing of MCA-induced sarcomas developed in *rag2*$^{-/-}$ mice that were subsequently rejected in wild-type counterparts revealed the expression of tumor-specific antigens, which elicited a high degree of reactivity by wild-type T cells (Schumacher & Schreiber, 2015).

The ubiquitous nature of somatic mutations in cancer would therefore suggest the potential for any tumor to become immunogenic. However, multiple investigations have confirmed that the vast majority of somatic mutations found in cancer do not lead to the formation of neoantigens recognized by autologous T cells (Lu et al., 2014; Linnemann et al., 2015; Schumacher & Schreiber, 2015). In fact, the majority of antitumor immune responses observed clinically actually target incidental, passenger mutations rather than transforming mutations (Linnemann et al., 2015). McGranahan et al. demonstrated another critical concept in the discussion of neoantigen repertoires by examining intratumoral heterogeneity in lung cancer. Indeed, the group revealed that tumors responding to checkpoint inhibition generally contained "clonal" neoantigens, or somatic mutations conserved throughout the tumor (McGranahan et al., 2016). Conversely, high degree of "subclonal" neoantigens was not associated with a clinical response, thereby suggesting elimination of neoantigen-containing cancer cells earlier in the course of tumor development, ultimately resulting in poorly immunogenic cancers despite the addition of checkpoint inhibitors (McGranahan et al., 2016). These observations support the concept of an equilibrium phase during which tumor cells evolve to be less immunogenic while conserving the mutational repertoire that fuels continued growth and metastasis.

Clinically, adaptive immune responses to solid tumors have generally been proportional to the degree of genomic instability associated with the tumor (Brown et al., 2014; Le et al., 2015a; Rizvi et al., 2015a). Accordingly, therapies designed to stimulate adaptive immunity have been most effective in melanoma, lung cancer and mismatch-repair deficient colorectal cancer; all of which are associated with high rates of somatic mutations relative to other malignancies (Alexandrov et al., 2013; Brahmer et al., 2015; Postow et al., 2015; Le et al., 2015a; Rizvi et al., 2015b). Building from these successes, prediction algorithms have been developed to identify neoantigens from nonsynonymous mutations within each individual tumor (Robbins et al., 2013; Duan et al., 2014; Linnemann et al., 2014). These algorithms have fostered the development of new therapeutic avenues designed to stimulate antigen-specific antitumor responses in a personalized manner.

On the other hand, it has been suggested that cancers with lower rates of somatic mutations may not develop sufficient neoantigens to generate autologous T cell responses. With the exception of a known subgroup of PC demonstrating microsatellite instability (Laghi et al., 2012; Wang et al., 2012), multiple independent whole exome sequencing efforts evaluating solid tumors demonstrated that PC has fewer mutations than melanoma or lung cancer (Alexandrov et al., 2013; Lawrence et al., 2013). However, conflicting results remain to be clarified when comparing PC to other solid tumors, such as prostate or breast cancer (Jones et al., 2008; Alexandrov et al., 2013; Lawrence et al., 2013). The possibility may exist that, regardless of advances in reversing local tolerance, the host may be unable to distinguish tumor from self. However, recent advances have challenged this notion. Allogeneic PC cell vaccinations have stimulated local immune responses and, in some cases, objective clinical responses (Lutz et al., 2014; Le et al., 2015b; Soares et al., 2015b). Specifically, vaccination has been shown to stimulate CD8 T cell responses to mesothelin, a protein of unknown biological function that is overexpressed in PC (Thomas et al., 2004). Adoptive transfer of mesothelin-specific T cells can target pancreatic tumors in a preclinical mouse model and HLA A2-dependent, mesothelin-specific T cell clones have been generated for use in human patients (Stromnes et al., 2015). Further, T cells isolated from patients with PC