Editorial

Cancer Prevention Research

Targeting "Retired Antigens" for Cancer Immunoprevention

Robert H. Shoemaker[1] and Thomas G. Forsthuber[2]

Abstract

Identification of immune targets for cancer immunoprevention, or immunotherapy, has historically focused on tumor-associated (self) antigens or neoantigens expressed on malignant cells. For self-antigens, overcoming tolerance can be a difficult challenge. Neoantigens do not suffer from this limitation, but the lack of recurrent mutations yielding common neoantigens that can be exploited in vaccines is a problem for many tumor types. Targeting "retired antigens," a specialized type of self-antigen, may have considerable advantages. Antigens no longer expressed in mature or aged individuals should pose reduced risk of autoimmune sequelae. Indeed, self-tolerance of these antigens may have naturally faded. Thus, when the retired antigens are highly expressed in cancer cells, it may be easier to overcome the remaining tolerance. Women who are BRCA1/2 carriers may be among the first to benefit as candidate retired antigens have been identified as highly expressed in ovarian and breast cancer cells. Although there is good preclinical data supporting this immune targeting concept, additional research is needed to understand the underlying immune phenomena and optimize the vaccine strategy. *Cancer Prev Res; 10(11); 607–8. ©2017 AACR.*

See related article by Mazumder et al., p. 612

In this issue of *Cancer Prevention Research*, the group of Dr. Vincent Tuohy reports targeting of the extracellular domain of anti-Müllerian hormone receptor II (AMHR2-ED) for effective preclinical ovarian cancer immunoprevention and treatment. Dr. Tuohy uses the term "retired antigen" to describe tissue-specific antigens no longer expressed as a normal feature of aging. Anti-Müllerian hormone acting via AMHR2 plays a key role in sex determination during early development and has a role in oocyte maturation during reproductive years. In postmenopausal years, AMHR2 is functionally retired. In addition to AMHR2-ED described here, Dr. Tuohy has previously targeted α-lactalbumin for breast cancer prevention (1). Both antigens are required for functioning of specific, differentiated tissues, show very limited expression in other normal tissues, but are frequently overexpressed in cancer cells derived from ovarian/fallopian tissues and mammary gland, respectively. This concept of targeting retired antigens for cancer vaccine development can be viewed as an extension of the testis-associated antigen-targeting concept. Antigens with very highly tissue restricted expression have long been recognized as appealing targets from the standpoint of minimizing potential autoimmune toxicities. Among others, therapeutic vaccines addressing the testis-associated antigen NY-ESO-1 have advanced to phase I/II clinical trials. Mazumder and colleagues show that tissue expression of AMHR2 is very limited in young individuals, that the expression of the external domain (ED) is exclusively limited to ovarian/fallopian tissue and that this expression is reduced with aging.

Clearly, the immunoprevention strategy proposed by Mazumder and colleagues must overcome tolerance to AMHR2-ED, a self-antigen, while at the same time avoiding undesired autoimmune phenomena. As this worked with both young and aged mice, this phenomenon was not dependent on the "retirement status" of the antigen. Interestingly, in young mice, there was no apparent effect on fertility, as one might expect upon vaccination and induction of autoimmunity against AMHR2-ED. Although at first, this may seem surprising, it is conceivable that tolerance mechanisms may have kept undesired autoimmune phenomena at bay in young mice, for example, by restricting tissue access or activation of autoimmune T cells at this "immune privileged" site, or via induction of regulatory T cells (Treg; ref. 2). The concept of immune privileged sites is one of the oldest immunologic tolerance phenomena, described over 100 years ago, and was first believed to be restricted to certain developmental periods, such as the neonatal period, or to affect particular tissues, such as the placenta, anterior chamber of the eye, and brain, and was thought to often depend on certain anatomic features, such as the blood–brain or blood–testis barrier or the anterior chamber of the eye (3). However, over the past decades, the concept of immune privilege has rapidly evolved from a being an anatomic and/or passive feature of specific sites only to an active strategy used by many tissues and with many different mechanisms and effector cells to protect themselves from excessive immune-mediated damage (3). It is telling that malignant tumors, in particular solid tumors, seem to have hijacked some of these key mechanisms to generate their own "tumor-associated" immune privilege, for example, by expressing immunosuppressive molecules, including FasL, PDL1, or immunosuppressive metabolites (e.g., the indoleamine oxidase pathway, IDO), or releasing immune-regulatory cytokines, such as TGFbeta (3). It is currently not fully understood how aging affects tissue immune privilege, but, conceivably, the ability to maintain local immune tolerance may be less efficient in older as compared with younger individuals, and therefore more easily permit the access and activation of immune cells, that is, elimination of tumor cells expressing "retired antigens." Although

[1]Chemopreventive Agent Development Research Group, Division of Cancer Prevention, NCI, Bethesda, Maryland. [2]Department of Biology, University of Texas at San Antonio, San Antonio, Texas.

Corresponding Author: Robert H. Shoemaker, NCI, 9609 Medical Center Drive, Bethesda, MD 20892. Phone: 240-276-7077; E-mail: robert.shoemaker@nih.gov

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immune privilege can be considered one of the "big three" immune tolerance mechanisms, the other two, that is, central and peripheral immune tolerance mechanisms may play a critical role as well in interpreting the results presented by Mazumder and colleagues. In fact, the physiologically higher expression of AMHR2-ED in younger mice may serve as a key protective mechanism in younger individuals by inducing and maintaining regulatory T-cell function, whereas upon "retiring" of this protein, Treg-mediated protection wanes and now opens the door for enhanced T- and B-cell–mediated anticancer immunity. Thus, in the realm of cancer immunotherapy and immunoprevention, targeting self-antigens that are not as well protected by immune-regulatory mechanism may have intriguing benefits. Obviously, the selection of "retired" self-antigens for immunoprevention will be a critical issue. Ideally, self-antigens selected for immunoprevention would be primarily expressed (i.e., "unretired") on cancer cells, but not in other tissues. However, it has to be considered whether "retiring" of self-antigens could be undone by biological processes, for example, during hormone treatment or inflammation. In situations where this might occur, the risk for autoimmune pathology may be increased, and the risks may outweigh the benefits of immunoprevention. The observation of vitiligo following treatment with some melanoma vaccines provides visible evidence of autoimmune destruction of normal cells sharing self-antigens with tumor (4). Last but not the least, Mazumder and colleagues showed the induction of anti-AMHR2-ED cellular and humoral immunity in several different mouse strains; however, in an "outbred" human population, it is conceivable that certain HLA haplotypes may not be as amenable to induction of immunity to the cancer vaccine. Thus, it may be a consideration to select more than one "retired" tissue antigen for the cancer prevention vaccine to assure protection across a broader human population and HLA spectrum.

Other key issues include the continued evolution of adjuvant strategies and improved antigen delivery to dendritic and other antigen-presenting cells to overcome immune-tolerance to self-antigens targeted by preventative cancer vaccines. Mazumder and colleagues show induction of strong Th1 and Th17 cell–mediated immunity, as well as antibodies, but not Th2 responses (i.e., IL5). For their preclinical studies, they used complete Freund's adjuvant, which is not suitable for clinical use. In contrast, commonly used human adjuvants for vaccination, such as aluminum salts, are notoriously poor at inducing cellular immunity and primarily induce mixed Th1/Th2 responses (5). Thus, aluminum salts are most likely not optimal for preventative cancer vaccines, and alternative adjuvants and/or routes of vaccination have to be considered and tested for use in human populations. What about a potential role for combination therapy using preventative cancer vaccines together with immune checkpoint inhibitors to enhance vaccine efficacy and enhance the potential to overcome tolerance? Although this may be a consideration, immune checkpoint inhibitors may increase the risk of severe adverse events in the future. Indeed, checkpoint inhibition as a single-agent intervention appears to have the potential to unleash a significant autoimmune reaction to "by-stander" antigens resulting in myocarditis and myositis (6). It may therefore be more desirable to proceed with careful vaccine design and delivery of precisely targeted retired self-antigens to reduce the risk of such sequelae.

Identification of AMHR2-ED as a new immune target for ovarian cancer chemoprevention could have major implications for women who are BRCA1/2 carriers. This high-risk cohort currently has few preventive options other than risk reduction surgery. Vaccination against AMHR2-ED, even if necessarily done after childbearing years, could provide a valuable option. Currently, little is known about the timing of the emergence of AMHR2-ED as a tumor-associated antigen. Presumably, this occurs very early in the carcinogenic process, as good evidence for cancer preventive efficacy was observed. Indeed, the results shown in Fig. 3C and D are among the most important findings in the current report. Dramatic delay and statistically significant inhibition of tumor growth were associated with increased survival of vaccinated animals. However, this observation is based on a small number of animals and a single experiment. The lack of a 100% effective result (there were no long-term survivors) may also be a matter of concern and may reflect the action of immune-editing. Tumor cells that downregulate AMHR2-ED may have a selective growth advantage and come to dominate the tumor cell population. The observation of rapid induction of therapeutic immunity in the mouse model (Fig. 3E) suggests that it may be possible to design a short-term clinical trial strategy where BRCA1/2 carriers planning risk reduction surgery are vaccinated and an effect on premalignant and malignant lesions is assessed in the surgical specimen. A further iteration of this strategy would be to create a bivalent vaccine targeting both AMHR2-ED and α-lactalbumin. In any event, independent confirmation of cancer-preventive efficacy and more detailed understanding of the underlying immune phenomena will be important for further development and optimization of this vaccine strategy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: R.H. Shoemaker, T.G. Forsthuber

Writing, review, and/or revision of the manuscript: R.H. Shoemaker, T.G. Forsthuber

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