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Spontaneous and Therapy Induced Immunity to Pluripotency Genes in Humans: Clinical Implications, Opportunities and Challenges

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Abstract

Recent studies have suggested that the core pathways regulating pluripotency in embryonal stem cells bear considerable overlap with oncogenesis. Here we discuss recent insights into the capacity of the human immune system to target some of the key pluripotency associated genes. Immunity to these genes is also induced in humans in the context of chemotherapy induced cell death in patients with germ cell tumors. Immunologic targeting of pathways associated with stemness has implications for both immune regulation of tumor growth as well as emerging regenerative therapies with embryonal stem cells.

Pluripotency, Stem Cells and Cancer

A major breakthrough in developmental biology and regenerative medicine in the past few years has been the induction of pluripotency in adult cells by forced expression of a limited set of genes (particularly SOX2 and OCT4) [1,2]. However these studies also indicate that the programs involved in the induction of pluripotency are intricately linked to cancer. Indeed formation of tumors is used as one of the criteria for evaluating the induction of stemness itself. Tumorigenicity of stem cells in regenerative medicine is directly proportional to their pluripotency. Interestingly, genes such as p53 which regulate oncogene-mediated induction of cancer also regulate the formation of such induced pluripotency stem (iPS) cells[3–6]. The presence of embryonal stem cell like gene expression programs can be detected in several human cancers and was found to correlate with adverse outcome in several databases of gene expression profiling in cancer [7–9]. Expression of these genes and/or their signatures also correlates with subtypes of cancers typically associated with aggressive clinical course, such as those with undifferentiated histology[8]. At least a proportion of these programs may be directly activated by oncogenes such as Myc, implicated in several human tumors[10]. Recently, more direct evidence linking pluripotency genes to cancers has also emerged. For example, aberrant expression of OCT4 is sufficient to induce tumors in mice[11]. SOX2 was recently identified as a common target of genomic amplification and a major lineage survival oncogene in patients with lung cancer[12,13]. Myc is already a well recognized oncogene. Pluripotency genes are also implicated in the concept of cancer stem cells[14]. These cells have typically been defined on the basis of their ability to seed tumors in animal hosts, to self renew and to spawn differentiated progeny. Several groups have documented the enrichment of embryonal stem

cell (ES) associated genes in cancer stem cells (CSCs), suggesting that these cells may utilize similar programs for self renewal[14]. Taken together, these emerging data suggest that pluripotency programs may be strongly linked to diverse cancers and serve as a potential therapeutic target in the setting of several cancers. This also raises a question as to whether these genes or pathways may be targeted via the immune system.

Spontaneous immunity to pluripotency genes: the case of plasma cell tumors

Evidence supporting the ability of the human immune system to mediate T cell responses against pluripotency genes came initially from antigen discovery approaches applied to cohorts of patients with plasma cell tumors. Multiple myeloma (MM) is a plasma cell tumor characterized by clonal expansion of plasma cells in the bone marrow, leading to lytic bone disease, infections, anemia and renal insufficiency. It is now appreciated that nearly all of MM is preceded by a long preneoplastic state termed monoclonal gammopathy of undetermined significance (MGUS)[15]. Interestingly, tumor cells in MGUS are known to carry many of the cytogenetic abnormalities identified in MM tumors, suggesting that interactions of tumor cells with the microenvironment may be a critical determinant of clinical malignancy in this tumor[16]. In prior studies, we have shown that T cells in MGUS patients can recognize antigens expressed on preneoplastic cells[17,18]. In order to better understand the nature of antigens recognized by the host response in the setting of MGUS, we analyzed the presence of antibodies against a large panel of tumor antigens using a serum antibody detection array[19]. These studies led to the surprising finding that the targets of host response in MGUS differed from those in myeloma. Interestingly, the top gene differentially targeted by the immune system in MGUS was the pluripotency gene, SOX2[19]. We then analyzed the presence of T cell responses against SOX2 using a peptide library spanning the entire protein. Using this assay, the presence of anti-SOX2 T cells could be detected in several patients with asymptomatic plasma cell tumors. Anti-SOX2 specific T cells consisted of both CD4+ as well as CD8+ T cells and could be expanded in culture using peptide pulsed autologous monocyte-derived dendritic cells (DCs). Importantly, these T cells readily responded to endogenously processed antigen on MGUS cells. The presence of naturally occurring T cell responses against SOX2 in patients with asymptomatic plasma cell tumors was predictive of an indolent course and markedly reduced the likelihood of progression to clinical myeloma requiring chemotherapy. Expression pattern of SOX2 also correlated with the putative clonogenic compartment in MGUS, and SOX2 specific T cells inhibited the clonogenic growth of MGUS cells in culture. In order to confirm these findings, we are currently evaluating the presence of anti-SOX2 T cells in the context of a large prospective SouthWest Oncology Group effort. Preliminary analyses from these patients have suggested that immunity to SOX2 is an independent predictor of outcome, and that patients with these T cell responses have significantly reduced risk of progression to clinical myeloma requiring therapy. The reason behind the lack of detection of anti-SOX2 T cells in MM needs further study. In view of the functional nature of the assay that we utilized, the possibilities relating to the lack of detection of these cells in MM in our studies include non-deletional mechanisms (including suppression and exhaustion)[20], and we are actively investigating these. While we have successfully been able to focus responses from peptide libraries onto single peptides in individual donors, this work has not yet been done in the context of HLA A2 donors.

Antibodies against SOX2 have also been observed in patients with lung cancer, wherein they correlate with improved outcome, although cellular immunity to this antigen in patients with lung cancer has not yet been examined[21]. Antibodies to SOX2 are also detected in patients with meningioma, a benign tumor with an indolent course in most patients[22]. Other investigators have recently made similar observations in the setting of MGUS. For

example, immunity to another developmental antigen OFD1 was detected in MGUS, but not in myeloma[23]. As with SOX2, OFD1 is also implicated in morphogenesis, although its role in carcinogenesis is presently not clear. Together these studies suggest the possibility that the nature of specific targets of spontaneous immunity may be predictive of clinical outcome in patients with cancer or more importantly, in otherwise healthy individuals or those with preneoplastic states. In particular, the presence of immune responses to pluripotency gene SOX2 may be predictive of a favorable course in cancer, particularly in MM.

Immunity to pluripotency genes in healthy donors

The finding that the human immune system can recognize antigens/epitopes derived from SOX2 encouraged us to look at immunity to other pluripotency genes, in particular against OCT4. As discussed earlier, both SOX2 and OCT4 are expressed together in ES cells and play a critical role in pluripotency of these cells. The expression of OCT4 in particular has been shown to mark putative cancer stem cells in several cancers. As with SOX2, we utilized an overlapping peptide library spanning the entire protein to screen for T cell responses against OCT4. Surprisingly, T cell reactivity against OCT4 derived epitopes could be readily detected in freshly isolated peripheral blood mononuclear cells from the majority of healthy blood donors[24]. The T cell response against OCT4 consisted of both CD4 as well as CD8+ T cells and resided primarily in the CD45RO+ memory T cell compartment. These T cells could be readily expanded in culture using dendritic cells loaded with dying OCT4 expressing teratocarcinoma cells, demonstrating that they are able to recognize antigen processed by antigen presenting cells. Taken together, these studies demonstrate that the human immune system is not tolerant to this critical pluripotency antigen. To the contrary, the facile detection of these responses in the memory compartment suggests the presence of a chronic immune response to this antigen in vivo in humans. In contrast to OCT4, we were unable to detect SOX2 reactive T cells using similar functional assays in freshly isolated T cells from healthy donors[19]. However, SOX2 specific T cells can be expanded from blood mononuclear cells of healthy donors after repeated restimulation with DCs loaded with dying germ cell tumors as a source of this antigen [24](and unpublished data). Therefore, for reasons that we do not fully understand, there is an apparent difference between patterns and properties of naturally occurring immunity to SOX2 and OCT4 in healthy donors.

Immunity to OCT4 in germ cell tumors

In addition to its critical role in the biology of ES cells, OCT4 has also emerged as a key oncogene in the setting of germ cell tumors (GCTs). Histopathologic studies have shown that GCTs commonly express OCT4, including carcinoma in-situ lesions. These findings are now encouraging surgical pathologists to utilize OCT4 expression in the clinical diagnosis of GCTs[25]. In order to better understand the changes in OCT4 immunity in the setting of an OCT4 expressing tumor in vivo, we analyzed patients with newly diagnosed GCT before initiation of chemotherapy[24]. In contrast to the findings with healthy donors, the detection of OCT4 specific T cells was significantly reduced in these patients. Importantly, the difference in immunity was specific for OCT4, as immunity to viral antigens in these patients was comparable to that seen in healthy donors. As noted previously, OCT4 specific T cells could be expanded in culture using dendritic cells loaded with dying teratocarcinoma cells. Access of dying tumor cells to antigen presenting cells could be a major pathway for the generation of these immune responses in vivo as testicular cancers typically lack expression of MHC II, and the OCT4 specific T cells are predominantly CD4+ T cells. Current chemotherapy of GCT with cisplatin based combination chemotherapy is very effective in inducing death of tumor cells. Therefore we analyzed the effect of chemotherapy

on the induction of anti-OCT4 T cell responses in vivo. In contrast to findings at baseline, most patients with GCT had detectable T cell responses against OCT4, indicating that these T cells could be activated and expanded in vivo. Together, these studies suggested that while T cell responses to OCT4 were deficient in patients with GCT, such immune responses were readily induced following curative therapy of these tumors. The capacity of chemotherapy to induce tumor immunity has potential implications for the capacity of these responses to contribute to the durable effects of chemotherapy.

Expression patterns of SOX2 and OCT4 in normal tissue

An important issue relating to targeting SOX2 or OCT4 is the pattern of expression and function of these genes in normal adult tissues. The early literature on pattern of OCT4 expression was complicated in part by misinterpretation of data due to the presence of pseudogenes and other caveats related to reagents and methods utilized [26,27]. More rigorous studies have reached the conclusion that OCT4 was not expressed in somatic tissues and stem cells [27–29]. More importantly, Lengner et al specifically targeted endogenous OCT4 locus by loxP sites [28]. OCT4 ablation in diverse tissues such as skin, bone marrow, intestine, and central nervous system did not lead to any detectable alterations. Together, these studies have provided compelling evidence that OCT4 is neither expressed nor required for somatic stem cell function. This expression pattern stands in distinct contrast to the common reexpression of OCT4 and activation of OCT4 target genes in the context of putative cancer stem cells in several cancers[7–9].

In addition to ES cells, SOX2 is thought to be active in embryonal brain and play a role in neural development in the eye and brain [30]. In the adult brain, SOX2 is commonly expressed in glial tumors[31]. The expression of SOX2 in the normal adult brain is less clear. One study found some SOX2 expressing cells in the ependymal layer[31], while another found weak expression in some cells in the Purkinje cell layer of the cerebellum[32]. SOX2 expression has also been described during development in other tissues such as neuroepithelial cells in olfactory epithelium[33], and Clara cell development in the lung[34]. We did not find expression of SOX2 in normal human hematopoietic cells including CD34+ stem cells[19], but the expression of SOX2 has been observed in osteoblast progenitors [35]. Taken together, these data suggest a potential developmental role of SOX2 in particular in some tissues of neural or neuroectodermal origin, however its function in particular in the context of adult tissues or stem cells is not clear. It is also worth noting that the nature of SOX2 dependent transcriptional activation in stem cells also depends on the co-expression of binding partners such as OCT4 or Pax6[36]. Here again, these data stand in contrast to common reexpression of SOX2 and SOX2 target genes in several cancers and emerging data about functional effects of SOX2 inhibition in tumor cells[7–9].

Other genes linked to ES cells and tumor immunity

Two other genes relevant to the biology of ES cells and under active investigation as targets of anti-tumor immune response are the tumor suppressor gene p53 and telomerase reverse transcriptase (TERT). However in contrast to the pluripotency genes discussed above, these genes are also commonly expressed in and are important for the function of nonmalignant cells, as well as adult stem cells in actively regenerating tissue such as hematopoietic tissue. Preclinical studies described anti-tumor efficacy of immunity against p53[37,38]. Both humoral and cellular responses against p53 can be detected in patients with cancer and early phase studies to harness these responses are ongoing[37,38]. Similarly, both naturally occurring and vaccine induced T cell responses against hTERT can be elicited in patients with cancer[39]. As the expression of these genes is not restricted to tumor cells, potential toxicity in terms of reactivity to normal tissues is a potential concern. Another class of

genes, cancer-germline antigens represent genes expressed predominantly in germ cells and a subpopulation of tumor cells, but not in normal tissues[40]. In contrast to those discussed earlier, these genes are not known to be important for pluripotency or stemness. It has been suggested that the expression of cancer-germline antigens may be enriched in putative CSC [41]. As we gain greater understanding of the biology of ES cells and particularly the link between pluripotency and cancer, it is likely that other antigenic targets of potential relevance to stemness and tumor immunity may emerge[42]. Of most direct interest is the possibility that other core ES pluripotency pathways such as nanog can also serve as targets of human immune response.

Clinical Implications and Opportunities

The finding of naturally occurring immunity to pluripotency genes such as SOX2 and OCT4 has several potential clinical implications. The detection of naturally occurring immune response may be predictive of outcome in the setting of precancer extends pioneering work with naturally occurring immunity in the setting of overt clinical cancer[43,44]. This appears to be particularly true in the setting of MGUS, wherein naturally occurring immunity against SOX2 was predictive of reduced risk of progression to clinical myeloma requiring therapy. Harnessing immunity to these antigens also holds promise in the setting of prevention or therapy of cancers. Anti-SOX2 T cells can inhibit the clonogenic growth of MGUS tumor cells [19]. T cell immunity against SOX2 and SOX6 is also being explored in the context of glioma stem cells[45,46]. Dendritic cell (DC) mediated targeting of neurospheres known to be enriched in CSCs led to greater anti-tumor immunity in mice compared to targeting bulk tumor cells[47]. Even injection of bulk ES or iPS cells has been recently explored as a strategy to induce immunity to ES associated genes and shown to induce protective immunity in a colon cancer model[48].

OCT4 is a particularly attractive antigen with regard to immunotherapy targeting CSCs. This is particularly of interest as T cell memory to this antigen can be readily detected in healthy blood donors[24]. OCT4 has been detected in putative cancer stem cells in several cancers, making it a candidate as a universal antigen targeting cancer stem cells. Naturally occurring immunity to this antigen therefore provides an opportunity harness it both in the context of vaccines, as well as adoptive T cell therapy in the setting of cancer.

Immunity to pluripotency genes also has clinical implications in the context of tumorigenicity of ES or iPS based therapies [2] [49]. The nature of tumors associated with ES or iPS therapy is not restricted to teratomas but includes diverse tumor types[50,51]. Indeed, all four of the core iPS factors are now strongly implicated in cancer. The tumorigenic potential of ES or iPS cells seems to be related to their differentiation status[52]. Therefore, one approach being taken to reduce the risk of tumors with stem cell based therapy is not to inject the stem cells themselves, but their differentiated progeny[49]. However this approach may reduce the very promise of stem cell therapy and still carries considerable risk, as differentiation is a dynamic and potentially bi-directional process, not an on-off switch. Harnessing immunity to pluripotency genes in this setting may allow elimination of the residual population of pluripotency competent cells in vivo, to help reduce the risk of tumors.

Challenges

Immune targeting of stem cell genes may also carry potential risks that should be addressed through careful clinical investigation. The most obvious risk relates to autoimmunity due to pathways shared with normal adult stem cells, particularly in actively regenerating tissues such as the hematopoietic system. Immune tolerance to pathways shared between CSCs and adult stem cells may also represent a potentially formidable challenge. In this regard, it is of

interest that at least some of the ES associated genes appear to be dispensable for the function of adult stem cells. One example is the pluripotency gene OCT4, which has been shown to be oncogenic in vivo, but is dispensable for the function of adult stem cells in mice [11,28]. SOX2 is known to be expressed by neural stem cells in mice [53] and therefore harnessing immunity against this antigen may carry a potential risk of neurotoxicity in the setting of developing brain. It is notable that no evidence of neurotoxicity has been noted in patients with MGUS or other tumors who have spontaneous T cell responses against SOX2 [19,21]. Immunity to both SOX2 and OCT4 is also induced in the context of curative chemotherapy of germ cell tumors in vivo in humans [24], and these therapies do not seem to cause obvious effects on normal adult stem cells. Appreciation of the importance of SOX2 as major driver oncogene in common cancers such as squamous cell cancer is now encouraging preclinical development of several pharmacologic approaches targeting this pathway [13]. Nonetheless, detailed preclinical investigation and careful assessment of potential risk/benefit in the individual clinical setting should be undertaken with stem cell targeted therapies, either immunologic or otherwise. In this regard, it is reassuring that at least some of the programs that regulate stemness seem to differ between adult stem cells and embryonal stem cells [28]. Further study of the role of these genes in the context of adult stem cells and somatic tissues is however indicated.

Summary

In this review, we have discussed new insights from our own work as well as others into the capacity of human immune system to respond to genes critical for pluripotency of ES cells. Alteration in immunity to these genes is observed in the setting of cancer. These genes may serve as critical targets for harnessing the immune system to control pluripotency in the context of regenerative medicine, as well as targeting cancer stem cells in diverse cancers.

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Abbreviations

ES	embryonal stem cells
MGUS	monoclonal gammopathy of undetermined significance
MM	Multiple myeloma
DC	dendritic cells
CSC	cancer stem cells
GCT	germ cell tumor
TERT	telomerase reverse transcriptase

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