

Review Article

Oncoantigens for an immune prevention of cancer

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Abstract: Vaccines are one of the main arms of preventive medicine. Recently a large series of experiments with cancer-prone genetically engineered mice have shown that preventive vaccines are also extremely efficacious inhibitors of the progression of carcinogenesis. Early vaccination affords significant and persistent protection, whereas its efficacy fades when neoplastic lesions become more advanced. Our current attempts to use combination strategies and technological advances to make vaccines effective in cancer prevention able to cure more advanced stages of cancer lesions are based on the temporary and systemic T_{reg} removal, the preparation of new bimodular plasmids for DNA vaccination, and the search for fresh target oncoantigens.

Keywords: Oncoantigens, anti-tumor vaccination, tumor immunoprevention

The promise of anti-tumor vaccines

Vaccines are one of the main arms of preventive medicine. Their efficacy rests on their ability to induce an effective antibody and cell-mediated immune response against a target antigen. Once triggered, immunity is kept at an efficacious protective intensity through natural restimulation or boosting [1].

Long lasting immunity and the polyclonal nature of the immune response elicited differentiate the protection afforded by a vaccine from the rapid, but short-lived protection induced by passive administration of a monoclonal antibody. However, despite these theoretical advantages, monoclonal antibodies alone have passed the acid test of clinical efficacy in cancer patients and are now emerging as an effective therapeutic strategy [2], whereas many trials using various vaccination strategies have shown vaccines have failed to become a new form of cancer therapy [3].

This depressing conclusion is somewhat lightened by some reports of their clinical efficacy in various neoplastic settings [4]. These results, along with approval by the FDA of an initial vac-

cine for cancer therapy [5] will certainly spur fresh and more rational strategies. The possibility of curing a cancer by activating the patient's immune response without dramatic side effects is still a fascinating goal. Its attainment is encouraged by the results of the countless vaccination-tumor challenge experiments on laboratory rodents performed in the last thirty years.

Other more recent experiments with cancer-prone genetically engineered mice have shown that vaccines are extremely efficacious inhibitors of the progression of carcinogenesis. For a review summarizing most of the studies of active immunoprevention of spontaneous autochthonous tumors in genetically-modified mice see [6].

The data emerging from these more realistic mouse models point to the use of vaccines in a conceptually new scenario, namely prevention of tumors in currently healthy persons at risk. Vaccination of the general population may be foreseen, as at present for the prevention of tumors caused by infectious agents [6]. In a more realistic perspective, several cohorts at risk could benefit from tumor-preventive vaccines, especially with regard to genetic risk, pre-

neoplastic syndromes, and individuals exposed to environmental carcinogens. In this context, impairment by a vaccine against an oncogene (ERBB2) of the progression of chemical carcinogenesis in hamsters is of particular interest. A new way may thus be found to treat healthy persons with a specific risk of a mutagen-induced cancer for whom no active therapeutic option exists at present (Cavallo et al, in preparation).

However, while vaccines for tumor prevention are a challenging future possibility, their exploitation to cure a cancer could provide a new answer to an urgent need. Here we describe our attempts to make vaccines effective in cancer prevention able to cure neoplastic lesions at progressive stages. Combinations and technological advances are exploited to enable a vaccine to control more advanced stages of cancer lesions.

Why do antitumor vaccines prevent cancer?

While the road toward vaccine truly effective in cancer therapy is still hard, experimental data show the efficacy of vaccines in tumor prevention. In a series of papers we have provided a somewhat conceptual manifesto on the rationale for the adoption of vaccines in tumor prevention [7, 8]. There are five key issues: 1) A truly preventive vaccine is administered to a healthy person before senescence, and thus in the absence of age-induced constraints on its ability to trigger an immune response; 2) Tumor-borne suppressive activities are obviously absent in a normal healthy person at risk; 3) Since no tumor is present, the specific immunity primed by a vaccine should be able to activate adaptive mechanisms towards a super-surveillance able to rapidly eradicate a mutated clone; 4) The low proliferation rate of indolent pre-neoplastic lesions cuts down the likelihood of selection of immune-resistant clones, while the vaccine-alerted immune response inhibits the lesion prior to complete neoplastic transformation; 5) Precancerous lesions are not yet sheltered from immune attack by the fibroblastic stroma, nor do they display either the tissue organization or the immunosuppressive abilities of large, proliferating tumor masses.

If these reasons suggest that preventive vaccination should be effective, overwhelming proof of its real efficacy is supplied by countless experimental data showing that laboratory rodents

can be preimmunized against almost all kinds of syngeneic tumors. Even tumors that in more conventional experiments fail to induce significant immune responses are promptly rejected when the recipient is effectively preimmunized [9].

This efficacy is even more evident in engineered mice with a genetic predestination to develop a lethal and invasive cancer [6, 10]. Balb/c female mice transgenic for the rat ERBB2 oncogene under the control of the Mouse Mammary Tumor Virus (BALB-neuT mice) are the model most studied in our laboratories [11]. In a distinct, stepwise fashion all these mice develop a lobular, invasive and metastatic mammary carcinoma in each of their ten mammary glands [12]. While in these mice T cells recognizing rat ERBB2 with high affinity are wiped out by central tolerance [13], anti ERBB2 vaccines of different kinds afford a significant protection against tumor onset [14-20]. Most of the protection rests on the direct and indirect action of the vaccine-elicited antibodies [15, 16, 21].

Here we will describe the potential and the limit of anti ERBB2 DNA vaccines made by plasmids encoding the extracellular and transmembrane domain of rat ERBB2 (RRT plasmids) electroporated intramuscularly [22]. **Figure 1** shows how effective two vaccine shots are in extending tumor-free survival (light red line). Sequential boosts every ten weeks maintained tumor free a

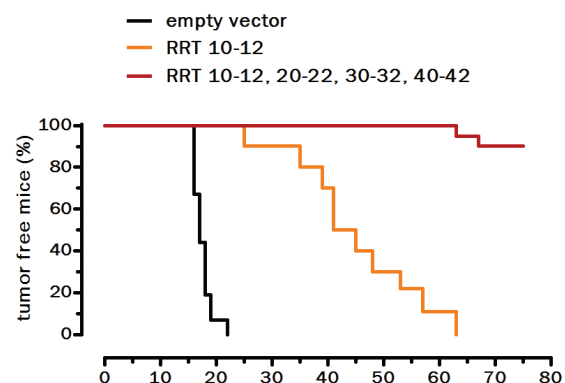


Figure 1. A DNA vaccine protects BALB-neuT mice from mammary cancer. When anti ERBB2 vaccine (RRT plasmids) is administered to young mice (weeks 10-12) it significantly extends their tumor-free survival (light red line) as compared to that of mice electroporated with the empty vector (black line). Vaccine boosting every 10 weeks maintained this protection during aging (dark red line).

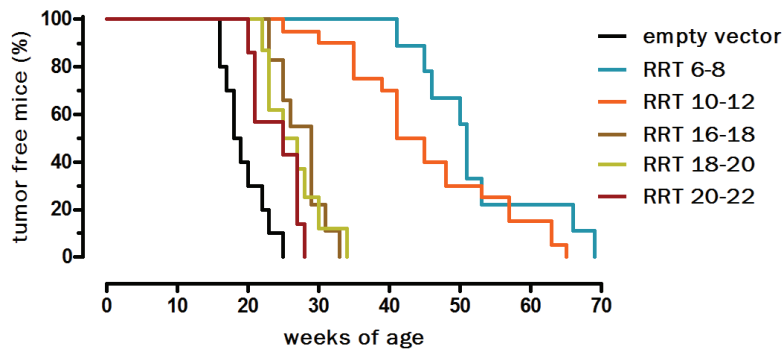


Figure 2. The DNA vaccine has a limited therapeutic potential in BALB-neuT mice. The protection afforded by the anti ERBB2 vaccine is very significant when administered to 6 to 8-week-old mice bearing areas of atypical hyperplasia, and progressively decreases when mice with in situ carcinomas (weeks 10-12), larger multifocal lesions (weeks 16-18), microscopic carcinomas (weeks 18) and more advanced invasive carcinomas (weeks 20-22) are vaccinated.

large number of aging mice (dark red line), a consequence of great importance when measured against the normal lifespan of these mice (rarely more than two years).

Figure 2 shows paradigmatically both the limit of this vaccination and prospects our current challenge: early vaccination affords significant and persistent protection, whereas its efficacy fades when neoplastic lesions become more advanced. Our current studies address the progressive difficulties encountered by the vaccine in protecting mice with advanced neoplastic lesions since we are persuaded that these data model a few of the difficulties that are encountered by vaccines when used in the therapy of human tumors.

Why cannot antitumor vaccines cure cancer?

The causes of this fading efficacy when neoplastic lesions become larger are of two kinds: 1) Difficulties of eliciting a strong immune response in an organism bearing a clinically diagnosed tumor; 2) Difficulties of the immune response elicited to penetrate the suppressive microenvironment at the outskirts of the tumor and attack its cells despite their stealthiness.

An established tumor acts like as a sponge that absorbs reactive T cells and suppresses their destructive function. Frequently tumor-associated lymphocytes infiltrating a tumor recognize tumor antigens but their effector activity is paralyzed by cytokines and other molecules in the tumor stroma [23]. In addition, a tumor commonly acquires the ability to become invisible to an immune attack through the loss or rarefaction of the expression on the cells membrane of the glycoproteins of the major histocompatibility complex (MHC) and alteration of

antigenic peptide processing machinery [24]. The inability of histocompatibility proteins to present the target antigen makes the T cell reaction ineffective and allows tumors to escape a key mechanism that would otherwise lead to their eradication.

Our recent work has been focused on ways of making a vaccine better able to elicit protective immunity in a tumor-bearing mouse on the assumption that a strong immunity will overcome most of the barriers made by a tumor's suppressive microenvironment and stealthiness. It has been repeatedly shown that a strong immune response, once elicited, can eradicate even large established tumors. Unfortunately, strong immune reactions against tumor antigens are difficult to elicit. Therefore, tumor debulking ability of strong immune responses elicited against minor histocompatibility antigens expressed by tumor cells can be adopted as proof of the principle [16, 25, 26].

Definition of the impediments to the induction of an effective immune response is the starting point for the elaboration of specific countermeasures. Usually a tumor has been growing for several years before being eventually diagnosed. During this period, preneoplastic lesions have had to overcome a series of surveillance mechanisms that have long kept them at bay. When this equilibrium phase breaks down, the most poorly immunogenic clones are those in the best position to escape and give rise to the tumor [27, 28]. Therefore, when a tumor reaches the clinical stage, it has already been edited as poorly immunogenic by the surveillance mechanisms [28].

Another important obstacle is presented by the expansion of suppressor cells that goes along

with tumor progression. Carcinomas frequently become able to secrete the granulocyte-macrophage colony stimulating factor [29], or the vascular-endothelial growth factor that cause the expansion of a population of myeloid immature cells that suppress the induction of an immune reaction [30]. In addition, through direct release of transforming growth factor (TGF)-beta, IL-10 and indoleamine 2,3-dioxygenase (IDO), or through activation of such secretions in myeloid-derived suppressor cells, tumor-associated macrophages and dendritic cells, a tumor converts naïve T cells into adaptive regulatory T (T_{reg}) cells that contribute to suppression of the induction of an immune response. Cunningly a growing tumor also exploits the reaction of natural T_{reg} cells.

Natural $CD4^+$ T_{reg} cells have the physiological function to keep immune tolerance to self-antigens and modulate the appropriateness of the immune responses. Most endogenous $CD4^+$ T_{reg} cells constitutively express the CD25 molecule (IL-2 receptor alpha-chain, IL-2R α) [31], while Foxp3 is the master gene that regulates their differentiation [32]. Over-expression of normal body proteins by tumor cells triggers their physiologic tolerogenic response: natural T_{reg} cells that recognize self antigens with high affinity are specifically activated and block both the expansion and function of effector T cells by reacting with the self antigen expressed by the tumor [33]. Therefore, proliferating and dying tumor cells provide a large amount of these tumor-associated self-antigens and thus trigger a feedback expansion of T_{reg} cells.

Both natural and tumor-induced T_{reg} cells inhibit the function of macrophages and dendritic cells by inhibiting the secretion of IL-12 and thus undermining their ability to promote activation and differentiation of effector T cells. In many cases the immune suppressor function of T_{reg} cells rests on their production of TGF-beta and IL-10, cytokines that inhibit the response of lymphocytes and macrophages [34]. Accumulation of T_{reg} cells at the tumor site and tumor draining lymph nodes hampers both the induction of a T cell response and the action of the effector T cells.

Besides the expansion of T_{reg} cells, a clinical tumor is endowed with many other immunosuppressive activities. It commonly releases a large array of cytokines that subvert the immune sys-

tem. Tumor-released GM-CSF leads to the direct expansion of immature myeloid cells. These cells not only help tumors to escape an immune response. They also aid in the construction of new blood vessels for tumor growth [35], while in the tumor microenvironment itself they may acquire a mixed dendritic cell - endothelial cell phenotype and promote tumor angiogenesis [36].

Dendritic cells are essential for the induction and maintenance of tumor-specific T-cell responses, both natural and induced by vaccines. Tumor-induced modulation of dendritic cell functions is one of the main factors responsible for tumor immune escape. There is clear evidence indicating that these defects are based on abnormal differentiation which results in decreased production of fully competent antigen-presenting cells and accumulation of immature tolerogenic dendritic cells [37]. Their dysfunction is caused by several tumor-derived factors reported in the gray area of **Figure 3**, right panel. Several of these molecules are also produced by dendritic cells from tumor bearing mice [37]. PGE₂, the major product of cyclooxygenase-2 (COX2), and IL-10 have similar immunosuppressive effects, for example they inhibit the production of IL-12 and the expression of co-stimulatory molecules by various types of dendritic cells [38]. Autocrine production of IL-10 is important in maintaining dendritic cells in an immature state. Moreover, secretion of the immunosuppressive cytokine IL-10 is one of the mechanisms by which T_{reg} cells control the immune response.

Current attempts to induce an effective immune response in tumor-bearing mice

Temporary and systemic T_{reg} removal

As tumor exploitation of regulatory mechanisms appears to be the principal cause of the poor ability of a vaccine to elicit a protective response in tumor-bearing mice, we explored the effect of T_{reg} removal alone and in combination with DNA vaccination against ERBB2. BALB-neuT mice are genetically predestined to undergo one of the most aggressive forms of metastasizing and lethal ERBB2-driven mammary carcinogenesis. As expected, during the progression of these mammary lesions, T_{reg} cell activity progressively becomes dominant, while T_{reg} cells expand in the spleen, tumor-draining

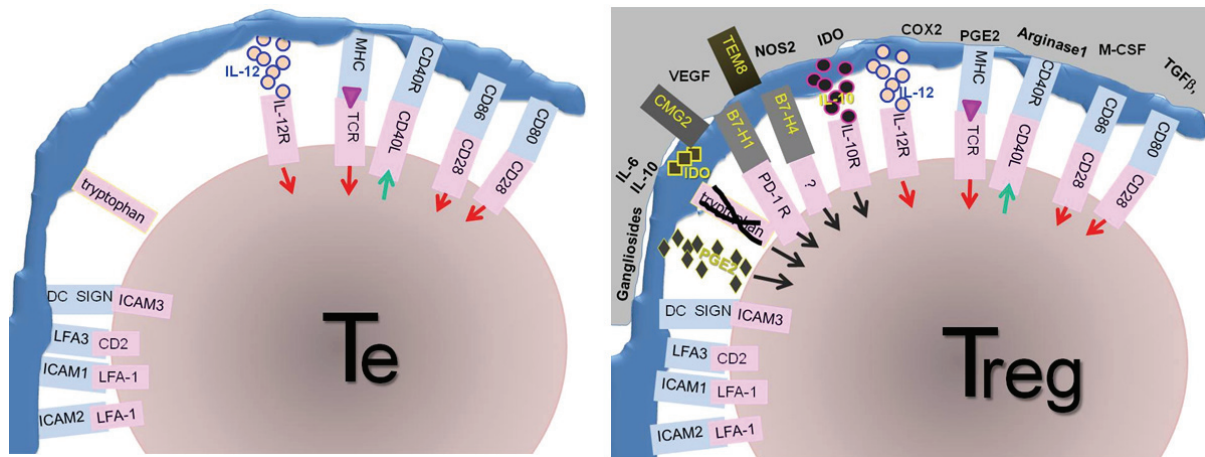


Figure 3. Presence of a tumor hinders induction of an immune response. Left panel: A dendritic cell (blue) expresses adhesion molecules that permit its first interaction with a T cell (pink). Following the binding with HLA glycoproteins presenting the antigenic peptide (fuchsia triangle) in their groove, the TCR transduces a series of activating signals that stabilize the interaction and lead to the activation of a T effector cell (Te). The TCR signaling is complemented by a series of costimulatory (CD80, CD86) and accessory (CD40) signals, and activating cytokines (IL-12) released by dendritic cells. Right panel: The presence of a tumor (in gray) and tumor produced molecules reported in the gray area modulates the function of dendritic cells. These start to overexpress COX2 and produce prostaglandins (PGE₂), overexpress IDO and inhibit the tryptophan metabolism, express suppressor costimulatory signals (B7-H1, B7-H4), produce less IL-12 while produces IL-10. The peculiarities of the signals delivered by tumor-disturbed dendritic cells lead to a preferential activation of T_{reg} cells.

lymph nodes, and tumor. Repeated administrations of anti-CD25 antibodies during the progression of carcinogenesis eliminate T_{reg} cells, extend tumor-free survival, reduce carcinoma multiplicity, and lead to the manifestation of a natural antibody and CTL-mediated reactivity against ERBB2 [39]. Loss of T_{reg} cells also causes the disappearance of immature myeloid cells. Although their absence may be due to the delayed carcinogenesis caused by T_{reg} cell removal, a crosstalk between these two regulatory cells cannot be ruled out [39].

As shown in **Figure 2**, when the lesions are initial and the tumor-elicited suppression is still negligible, the anti-ERBB2 vaccine prevents their progression, whereas its efficacy fades as they progress, and is almost nil against diffuse invasive microscopic tumors. Combination of anti-ERBB2 vaccination with temporary T_{reg} removal elicits a protective response at progression stages at which the vaccine alone is ineffective (**Figure 4**). This rescue of a vaccine's protective potential goes along with enhanced antibody production and activates a latent population of cytotoxic CD8⁺ T cells expressing a cryptic T cell receptor (TCR) repertoire [40]. These results underscore the absolute impor-

tance of using combined vaccine strategies when dealing with the induction of a protective immune response in tumor-bearing mice.

Bimodular plasmids for local relief from suppression

These experiments show how intricate interactions between regulatory mechanisms and auto-

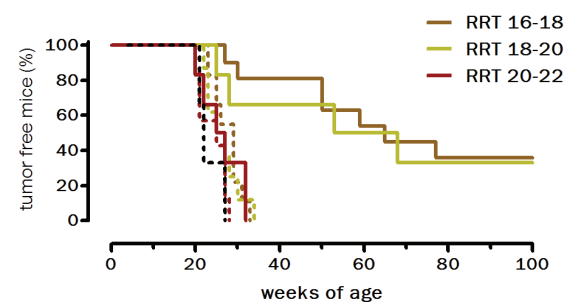


Figure 4. Temporary T_{reg} depletion enables anti-ERBB2 vaccination to inhibit established carcinomas. Mice bearing large multifocal lesions (weeks 16-18), microscopic carcinomas (weeks 18-20) and more advanced invasive carcinomas (weeks 20-22) were vaccinated with ERBB2 plasmids alone (dotted lines) or following T_{reg} removal (continuous lines).

immune-based immunosurveillance are. This coexistence is both intriguing and alarming, because in the long run maneuvers leading to T_{reg} cell removal, instrumental for the induction of an efficient antitumor reactivity, may also trigger significant autoimmunity to self-antigens. It is therefore essential to find a way to inhibit suppressor mechanisms only when the tumor antigen has to trigger host immunity.

By exploiting what has been shown by Yen et al. [41], we are studying the efficacy of plasmids of a new kind to be used as DNA vaccines capable of activating a better immune response when the regulatory or suppressor mechanisms induced by a tumor must be overcome. These plasmids are formed of two modules. The first codes for the conventional antigen against which the immune response is addressed, while the second transcribes one or more shRNA to neutralize a few of the suppressor mechanisms that hamper the induction of an effective immunity in antigen presenting cells. By inhibiting the negative regulatory mechanisms that interfere with a cell's ability to present the antigen, these shRNA facilitate efficacious presentation of the antigen coded by the plasmid. By contrast, with systemic removal of regulatory and suppressor mechanisms on the part of conventional treatments, these bimodular plasmids only remove suppression in the cells they enter, that is to say only in those presenting the antigen coded by the plasmid. This selective inhibition concerns only a small number of cells directly involved in induction of the immune response. It is thus free from the serious autoimmune phenomena that may follow systemic blockade in all the immune system compartments, of the important and physiological regulatory and suppressor molecules.

Characterization of the right target

Tumor antigens most commonly targeted by therapeutic vaccines in the past were those recognized by T cells, such as those from the MAGE family antigens, tyrosinase and related proteins [42]. However, in a preventive setting these antigens do not look like suitable targets. Their recognition by T cells rests on the presentation by MHC glycoproteins while their presence is not required for the progression of neoplastic transformation. When neoplastic progression occurs in the presence of an antigen-specific immune attack, clones with a poor ex-

pression of antigen peptides by MHC glycoproteins or lacking the target antigen are those that are positively selected.

Since an ideal tumor antigen that does not induce autoimmunity and cannot be negatively selected probably does not exist, we have to come to suitable compromises. Perhaps, the vaccine induction of a cell or tissue-specific autoimmune response may be considered acceptable when it allows the prevention of or cures cancers arising from nonessential cells or organs, such as melanocytes and breast, prostate, thyroid, and testis [43-45]. However, such targeting of widely diffuse auto-antigens is raising many concerns related to confinement of the vaccine-induced autoimmunity and the extent to which autoimmune impairment of more or less crucial body functions is ethically acceptable in order to cure a cancer or –even worse– to reduce a distant risk [46].

A softer compromise is to target self molecules that have an essential role in the progression of carcinogenesis. We have called these molecules “oncoantigens” [47]. The rationale of this compromise is threefold: 1) Since oncoantigens play a role in tumor growth their loss will impair or block tumor progression. Inevitably, oncoantigen-less clones are negatively selected; 2) Oncoantigens are commonly overexpressed at the tumor site. An immune response elicited against an oncoantigen is indeed a form of autoimmunity. With a few of these oncoantigens, the risk of a generalized epitope-spreading and autoimmunity is limited by their selective overexpression at the tumor site. An ideal oncoantigen displays a low level of expression in normal cells and high levels in transformed cells and their microenvironment; 3) Oncoantigens can be attacked by T cells and by antibodies, depending on the place of their expression. Class I oncoantigens are expressed on the cell surface and are targeted by antibodies and T cells (receptors, adhesion molecules, etc); Class II oncoantigens are present in the tumor microenvironment and targeted by antibodies (growth factors, angiogenic factors, etc) [48]; Class III oncoantigens are intracellular proteins that can be targeted by T cells only (non-receptor tyrosine kinases, transcription factors, cell cycle molecules) [6].

Since class I and II oncoantigens can be inhibited by antibodies, the immune response elicited by a vaccine is not disallowed by the rare-

faction of MHC glycoprotein expression. Antibodies to Class I can directly block the ability of a receptor to bind its ligand, form dimers with other receptors, and remain on the cell membrane. The resulting signaling blockade might lead to the inhibition of cell proliferation, and the activation of apoptotic programs. In addition, antibodies to Class I oncoantigens can indirectly recruit immune functions such as complement-mediated cytotoxicity and antibody dependent cellular cytotoxicity. The activation of complement can also guide the activity of macrophages, granulocytes and natural killer cells, and play a pro-inflammatory role. The activity of Class II oncoantigens can also be directly neutralized by antibodies that thus inhibit biological activities essential for tumor expansion such as the recruitment of inflammatory cells and tumor angiogenesis. Class III oncoantigens are probably not susceptible to antibody-mediated reactions, but only to T cells.

The same oncoantigen may belong to different classes. The receptor tyrosine kinase ALK is a Class I oncoantigen when expressed on the surface of solid tumors, whereas in anaplastic large cell lymphomas the NPM-ALK translocation converts it into an intracellular Class III oncoantigen. The idiotype of B cell neoplasms is either a secreted immunoglobulin, a surface Class I oncoantigen or an intracellular Class III immunoglobulin [6].

The search for fresh oncoantigens

Many experimental studies with cancer-prone mice genetically engineered in many ways have consistently illustrated the potential of vaccines addressing Class I [6, 10] and, to a lesser extent, Class II [47, 48] oncoantigens. This robust experimental evidence is spurring the hunting, high and low, for fresh oncoantigens to be targeted by more effective vaccines. Progression through carcinogenesis requires the activation of specific gene and regulatory pathways, and may be blocked when these are selectively perturbed. Characterization of Class I and Class II oncoantigens differentially expressed during the early stages of carcinogenesis leads to the identification of prominent pathways and the molecules against which to trigger an immune response. This provides an unprecedented opportunity to trigger a definitive or long-lasting protection against incipient cancer [49]. Even incomplete tumor eradication leading only to an

oncoantigen-based immune editing and resulting in no more than the selection of oncoantigen-less clones may dramatically impair tumor progression.

But how can hunting for oncoantigens be planned? We first looked for tumor and microenvironment-associated oncoantigens by comparing gene signatures of different stages of tumor progression in cancer-prone transgenic mice carrying a defined genetic alteration relevant to human cancer. We then looked to see whether the mouse oncoantigen has a human ortholog. Only subsets that have an ortholog in humans and are overexpressed by human tumors are selected. Lastly, the immunogenicity and potential protective role of the selected mouse putative oncoantigens is assessed in mouse experiments of preventive vaccination. The protective potential of the immune response elicited is evaluated in cancer prone transgenic mice in their distinct cancer stages [47, 49].

We are convinced that this strategy provides groundwork for the rational design of cancer vaccines for clinical trials. Microarray transcription studies are a powerful instrument to identify potential oncoantigens on a genome-wide basis. Results obtained from such studies can always be validated, at the protein level, against normal and neoplastic tissues by mining protein databases (www.proteinatlas.org). We use genome-wide transcriptional analysis on tumor samples collected from cancer prone-transgenic mice with progressive stages of tumor. Gene expression profiles are generated and microarray data analysis is performed with a consolidated pipeline [47] and software [50].

A fresh oncoantigen identified in a mouse model is of great interest only if it has a similar role in human cancer. Therefore, we determine whether the expression of these proteins is low in normal human tissues, but high and homogeneous in human cancers. Mouse oncoantigens overexpressed in human cancers are then re-validated in the mouse model by assessing their immunogenic and protective potential. Limited expression in normal tissue and high expression in tumor specimens are the hallmark of an attractive oncoantigen. To limit the risk of widespread autoimmunity it is important to select those with a limited expression in normal tissues. Class I oncoantigens expressed on the

cell membrane are theoretically the most promising for vaccination, since they can be the target of both cell and antibody-mediated immune responses. Class II oncoantigens present in the tumor microenvironment, such as extracellular matrix proteins or cytokines, can be an effective target for neutralizing antibodies. Class III oncoantigens are an appropriate target only when their peptides are suitably presented by MHC glycoproteins on the tumor cell surface.

Oncoantigens expressed by cancer initiating cells (CICs)

Identification of oncoantigens expressed during the early phases of cancer allows vaccination against molecules delivering signals that are critical in a specific stage of cancer progression [47]. However, it has become evident that many human malignancies are organized in a hierarchical network consisting of a few slowly dividing CICs, rapidly dividing amplifying cells, and differentiated tumor cells [51]. The stem-cell-like properties of CICs (self-renewal and re-establishment of tumor heterogeneity) place them at the top of this hierarchy [52], and make them responsible for tumor progression, metastasis, resistance to treatment, and recurrence [53, 54]. The identification of an oncoantigen selectively expressed by CICs may lead to the identification of oncoantigens that may be a very effective target for an immune attack. Moreover, an immune response against an oncoantigen overexpressed by CICs but absent or poorly expressed by adult normal tissues would further limit the risk of widespread autoimmunity.

Our current data show that mammary carcinomas spontaneously arising in BALB-neuT mice contain a population of sphere-generating cells endowed with the ability to initiate tumor *in vivo* [55]. Comparison of transcription profiles of a carcinoma-derived cell line cultured as an epithelial monolayer or as mammospheres in serial passages with human breast tumors and normal human tissue microarray data sets identifies a transcription signature associated with CICs. Examination of this signature is leading to the isolation of a set of transcripts whose expression increases in the cells of the succeeding passages of the mammospheres. Identification of fresh oncoantigens expressed by CICs could provide the opportunity for targeting molecules delivering signals crucial for cancer

stemness, a crucial issue in the elaboration of more effective anti-tumor treatments (Cavallo et al., in preparation).

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