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BRIEF REVIEWS

Unique Human Tumor Antigens: Immunobiology and Use in Clinical Trials¹Giorgio Parmiani,² Annamaria De Filippo, Luisa Novellino, and Chiara Castelli

The individual, unique tumor Ags, which characterize each single tumor, were described 50 years ago in rodents but their molecular characterization was limited to few of them and obtained during the last 20 years. Here we summarize the evidence for the existence and the biological role of such Ags in human tumors, although such evidence was provided only during the last 10 years and by a limited number of studies, a fact leading to a misrepresentation of unique Ags in human tumor immunology. This was also due to the increasing knowledge on the shared, self-human tumor Ags, which have been extensively used as cancer vaccines. In this review, we highlight the biological and clinical importance of unique Ags and suggest how they could be used in clinical studies aimed at assessing their immunogenic and clinical potential both in active and adoptive immunotherapy of human tumors. The Journal of Immunology, 2007, 178: 1975–1979.

A milestone in the history of human tumor immunology is certainly the molecular characterization of the first human melanoma Ag (MAGE)³ recognized by T cells (1). Nowadays, this Ag is known to belong to the group of cancer/testis or germinal Ags, which are expressed by histologically different human tumors and by a limited subset of normal cells of testis and placenta.

In 1993, we first described self-differentiation Ags shared between normal melanocytes and melanoma cells and recognized by class I HLA-restricted T lymphocytes (2); a year later, the first of these Ags was molecularly characterized simultaneously by the groups of Boon and Rosenberg (3, 4) and named Melan-A and melanoma Ag recognized by T cell-1, respectively.

Important papers published by both these research groups then showed that in vitro generation of CTL from peripheral blood of melanoma patients, endowed with the ability to recognize self-Ags, was possible (5). These results stimulated a race to transfer the use of such Ags in clinical trials aimed at assessing the in vivo immunogenicity and clinical efficacy of vaccines

based on HLA class I peptide epitopes derived from these Ags. Subsequently, other similar tumor Ags have been discovered belonging to the same families, i.e., cancer/testis and differentiation Ags (6), and new vaccine formulations containing these Ags in the form of peptides, proteins, and recombinant DNA were tested in patients affected by different neoplasms, including melanoma, renal cell carcinoma, and a variety of epithelial tumors. Altogether, the clinical outcome of these trials was limited (7, 8), despite the ability of some Ags and their combination to generate a high frequency of T cells against the vaccine (9). It was recently found that, among other reasons, the low affinity of self-Ags may impact on their in vivo immunogenicity. Moreover, such self-Ags can trigger functional regulatory T lymphocytes that would interfere with the expansion of CTL (10). Therefore, even when T cell responses are detectable at high levels in the blood or in tumor tissue, their efficacy in inducing a shrinkage of tumor masses remains rather weak and clinically unsatisfactory (9). In addition of being weakly immunogenic by themselves, these Ags can activate a series of tumor immune escape mechanisms or immune dysfunctions that cause an even lower antitumor effect (11).

Unique Ags and their main features

However, we believe that a major issue in tumor immunotherapy that has only recently rekindled the attention of tumor immunologists is the nature of the Ags that have been used in clinical trials of vaccination and adoptive immunotherapy, i.e., the cancer/testis and differentiation self-Ags. In fact, early studies of tumor immunology in animal models indicated that the Ags involved in the rejection of tumors, either transplanted (12) or primarily induced by chemicals or UV rays (13, 14), were the unique Ags. Such Ags characterize each single neoplasm and were shown to be diverse between two tumors induced in the same animal or even in different tissue fragments of the same tumor nodule (15, 16). Nowadays, we know that such Ags are by and large the results of somatic point mutations occurring in many different proteins expressed by tumor cells (17), and therefore, they represent the only true, tumor-specific Ags not expressed by any normal tissue. Other possible but less frequent mechanisms for generation of these Ags, such as alterations in

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³ Abbreviations used in this paper: MAGE, melanoma Ag; CDK, cyclin-dependent kinase; HSP, heat shock protein.

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RNA splicing, have been reported (18, 19). An important additional feature of unique Ags is their potential resistance to immunoselection in cases when the mutated protein is crucial to the oncogenic process and thus indispensable for maintaining the neoplastic state or because functionally involved in fundamental pathways of cell survival. Immune response against unique, mutation-derived Ags can also be viewed as a potential control of the genome integrity by the immune system that is theoretically equipped to recognize and eliminate cells bearing harmful mutations (20).

Since the race for obtaining a clinical result with the available shared, self-Ags first discovered in human tumor cells went on for a decade or so, almost no attention was paid to individual Ags of human tumors as potential targets of both active (vaccination) and adoptive immunotherapy. Admittedly, this was also because of the contention that unique Ags would have been difficult to use in the clinic owing to the lack of rapid methods for their identification and molecular characterization at the single tumor/patient level. Moreover, as in the mouse system, a single human tumor can express multiple unique Ags and generate new ones during progression (21), making their characterization even more cumbersome.

In the last few years, however, the situation has changed, albeit slowly. In fact, the first molecular description of a T cell-defined unique human melanoma Ag, resulting from a point mutation of cyclin-dependent kinase (CDK4), was reported in 1995 (22). Subsequently, sporadic publications described the expression of such Ags as epitopes recognized by T cells in the context of both class I and II MHC in other human tumors such as non-small cell lung cancer, bladder cancer, renal cancer cells, head/neck cancer, and melanoma (Ref. 6 and Table I).

Immunogenicity of unique Ags

A critical issue for tumor Ags is their immunogenicity that can be assessed in vitro or ex vivo by a variety of techniques. Once molecularly characterized, each of these unique Ags could be

tested to see how strong was its ability to elicit an Ag-specific T cell response after in vitro stimulation of patients' PBMCs or by an ex vivo assessment of the PBMC response of untreated or immunized patients. While evidence for a response stronger than that of shared self-tumor Ags in PBMCs of the same patient simultaneously stimulated by unique and shared peptide Ags from the same tumor was not clearly demonstrated and only occasionally tested, an analysis of the natural response of melanoma patients against shared and unique Ags expressed by the autologous tumor revealed a predominance of the latter during the favorable course of the disease both for class I and class II HLA-restricted Ags (21, 39). In fact, cancer patients rendered disease-free by surgery or chemotherapy maintained a strong activity against unique Ags over time (21, 39, 40) while that against self-common Ags faded away in absence of tumor burden (41). Moreover, T cell populations used in adoptive immunotherapy of melanoma patients and associated with tumor regression were shown to be directed against mutated Ags that include HLA-A11 class I gene protein and frameshifted products of the *CDKN2A* tumor suppressor gene locus (42).

Although no vaccination or adoptive immunotherapy trials deliberately targeting molecularly characterized unique tumor Ags have been conducted thus far, attempts have been made to bypass such a drawback by immunizing with the potentially whole repertoire of autologous tumor cell Ags, including the unique ones.

This has been accomplished by using irradiated, cytokine gene-transduced autologous tumor cells (43), tumor lysates (44), heat shock proteins (HSPs) deriving from autologous tumors (45), hybrids between autologous tumor, and dendritic cells or amplified tumor-derived RNA (46). An immune response to unique Ags that was found to be associated to a clinical benefit has been described in patients with renal cancer cells vaccinated with GM-CSF gene-transduced tumor cells (43) and patients with melanoma as indirectly shown from the analysis of clonal T cell response in patients immunized with a

Table I. Unique human tumor Ags recognized by class I and class II HLA-restricted T cells

Ag	Abbreviation	Tumor	Reference
Class I HLA-restricted T cells			
Cyclin-dependent kinase 4	CDK-4/m ^a	Melanoma	22
Melanoma ubiquitous mutated 1, 2	MUM-1/2	Melanoma	18
Melanoma ubiquitous mutated 1, 2	MUM-1/2	Melanoma	23
Melanoma ubiquitous mutated 3	MUM-3 (<i>Helicase</i>)	Melanoma	24
β -Catenin-mutated	β -Catenin-m ^a	Melanoma	25
Myosin mutated	Myosin/m	Melanoma	26
Redox-perox mutated	Redox-perox/m	Melanoma	27
Melanoma Ag recognized by T cell-2	MART-2/m	Melanoma, lung small cancer cells	28
β -Actin-4 mutated	β -Actin/4/m ^a	Non-small cell lung cancer	29
Elongation factor 2	ELF2-M	Non-small cell lung cancer	30
Tumor draining lymph node	TDL	Non-small cell lung cancer	31
Caspase-8 mutated	CASP-8/m ^a	Head and neck	32
HLA-A2 mutated	HLA-A2-R170J	Renal cancer	33
Heat shock protein 70-2 mutated	HSP70-2/m ^a	Renal cancer	34
Cyclin-dependent kinase N2A	CDKN2A	Melanoma	42
Class II HLA-restricted T cells			
Cell division cycle 27	CDC27 ^a	Melanoma	35
Triosephosphate isomerase	TPI	Melanoma	36
Low-density lipid receptor/GDP-L-fucose: β -D-galactosidase 2- α -L-fucosyltransferase	LDLR/FUT	Melanoma	37
Fibronectin mutated	Fibronectin/m	Melanoma	38
Receptor-type protein-tyrosine phosphatase K	RT-PTP-K/m ^a	Melanoma	39

^a Proteins likely involved either in maintaining the neoplastic condition or in the progression and metastatization of cancer cells.

MAGE-A3 peptide (47). In fact, Lurquin et al. (47) reported that vaccination of melanoma patients with MAGE-A3 can generate clonal T cell responses against Ags not included in the vaccine but expressed by patient tumor cells, including unique Ags, through the mechanism of "antigenic spread." The most compelling case in favor of the strong immunogenicity of unique Ags and of an association between anti-unique Id-specific Ag response and clinical outcome is that of non-Hodgkin's lymphoma patients vaccinated with their own Id protein under different formulations (48, 49). T cell reactions against multiple unique epitopes were documented and found to be associated with molecular remission in a significant fraction of patients (48).

Implications for immunotherapy

Potential mechanisms why unique Ags specific immunity may be clinically more effective than shared Ag-specific immunity may lie in 1) the exquisite tumor specificity, 2) lack of any possible form of tolerance as compared with shared Ags, 3) multiple expression by a single tumor, and 4) resistance to host immunoselection being unique Ags essential to the maintenance of the neoplastic conditions. How to design clinical trials that preferentially boost the immune response targeting truly tumor-specific unique Ags? While this is relatively easy and already possible for tumors of hematological origin as B cell lymphomas whose altered Ig Id can be sequenced and used as patient-specific unique tumor Ag (48, 49), it represents a quite difficult task for solid human tumors. Obviously, the ultimate strategy for targeting such types of Ags will imply sequencing of the whole genome of each individual tumor followed by the selection of mutated peptides whose motifs are predicted to be presented by the HLA alleles of the patient bearing that particular mutated tumor. This approach will potentially include all the mutated Ags expressed by that tumor at the time of analysis, but it surely requires a massive but not impossible effort in cancer genome analysis (50). However, while firmly keeping the principle of using molecularly defined, unique, tumor-specific Ags for vaccination, in the near future a feasible strategy may be proposed that limits such an analysis to a defined set of genes known to be selective targets for mutation in a tumor of a given histology (50). Although in only one case the mutation generating a unique melanoma Ag has been proven to have a direct role in tumor metastasis (37), in the majority of unique Ags, the mutations generating the immunogenic epitopes occurred in genes whose functions were relevant for tumor viability and progression (e.g., *CDK4*, *CDKN2A*, *PTRRK*, *CASP8*, etc.; see Table I) (6). Starting from this observation, mutation analysis could be performed for those genes belonging to the signal pathways known to be activated or in genes known to work as oncosuppressors for that particular tumor. Targeting such type of immune response, directed toward proteins crucial for tumor cell survival, may have a better chance to lead to a clinical benefit. In fact, proteins essential in cell survival or involved in maintaining the transformed phenotype, are likely to be preserved during the evolution of the disease despite the Ag loss variants that may occur under the selective pressure of the immune response.

Alternative strategies could be considered to selectively enrich the vaccine for tumor-mutated vs not-mutated proteins although the resulting product may not have a molecularly defined composition. Taking advantage of new molecular tech-

niques such as heteroduplex formation (51), strategies could be developed for selecting altered, mutated genomic sequences from which a library potentially enriched for altered transcripts should be constructed and then used to produce pools of mutated proteins expressed by the original tumor.

An additional vaccine potentially enriched in mutated epitopes can be made from autologous tumor-derived HSPs. HSPs are a large family of proteins with different intracellular localization and endowed with crucial functions in maintaining cell homeostasis such as folding and translocation of newly synthesized polypeptides in different subcellular compartments (45). Among HSPs, HSP70 and Grp94/gp96, due to their peptide chaperone activity and their ability to actively interact with professional APCs, also display crucial immunological functions (52). These proteins have been identified as tumor-rejection Ags and described as responsible for the induction of a protective anti-tumor T cell response mediated by unique Ags in murine tumors. In fact, studies in rodents showed that prophylactic and therapeutic vaccinations with purified preparations of HSPs isolated from tumor cells leads to protective immunity against the cancer used as the source of the HSP but not against other syngeneic neoplasms (52). These data strongly indicate that vaccination with HSPs can elicit a T cell response directed against unique, mutated Ags implying that mutated peptides are therefore actively chaperoned by these proteins (53).

As for the human system, HSPs purified from human tumors or virus-infected cells have been shown to induce a CTL response in vivo and in vitro against a variety of Ags expressed by the cells from which these immunogenic proteins were purified, confirming that the HSP immunological properties are fully maintained in the human setting (54, 55). In fact, using an autologous system, we and others (54–56) were able to show that in vitro stimulation of patients' PBMCs with melanoma-derived Hsp70 and Gp96 elicited tumor-specific T cells and that these T cells included effectors directed not only against melanoma-shared Ags but also against an individual Ag generated by a mutation occurring in the tyrosine phosphatase receptor protein (receptor-type protein-tyrosine phosphatase K) (Ref. 39; C. Castelli, unpublished data). The ability of tumor-derived Gp96 to induce a tumor-specific response in vivo when used as a vaccine has been confirmed in metastatic melanoma and colorectal cancer patients (57, 58). However, due to the intrinsic difficulties in obtaining enough autologous tumor cells, the ability of boosting in vivo T cell responses directed against unique Ags still remains to be fully documented in the human HSP system.

In conclusion, although the construction of a tailored, personalized vaccine for each single patient based on unique Ags remains a difficult task, efforts should be made to overcome the present difficulties. Time is ripe for a coordinated effort to use unique Ag in cancer immunotherapy. We predict that technological advances will make the molecular characterization of unique Ags feasible in a short time, thus allowing their immunotherapeutic targeting to be tested in clinical trials.

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