OncoImmunology 1:5, 579–580; August 2012; © 2012 Landes Bioscience

Can the exome and the immunome converge on the design of efficient cancer vaccines?

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Human cancers carry hundreds of non-synonymous mutations, several dozens among which may lead to the generation of tumor-specific MHC Class I-restricted epitopes. Hence every patient's tumor harbors a highly specific mutational and antigenic signature and up to 95% of these mutations are unique. This "mutanome" can be identified by deep sequencing and can be subjected to systematic analyses of the immunogenicity of mutated proteins/peptides. We anticipate that this approach will lead to individualized immunotherapies by means of tailored vaccines.

The "cancer epitope landscape" was conceptualized by Vogelstein and Allison who applied an in silico-based epitope prediction algorithm to high throughput sequencing data to identify candidate tumor antigens in breast and colon cancers.[1] This approach led to the estimation that individual cancers harbor an average of 7–10 unique HLA-A*0201 epitopes, some of which may correspond to mutations in proteins that "drive" the oncogenic process (as opposed to functionally irrelevant "bystander" mutations). Recent methodological progress suggests the potential exploitation of this kind of approach to optimally design efficient cancer vaccines.

By cloning a cDNA library derived from normal human prostate into the oncolytic vesicular stomatitis virus (VSV)—termed altered self antigen and epitope library (ASEL)—Richard Vile and coworkers could cure the vast majority of mice bearing established prostate cancers.[2] Indeed, the authors performed repetitive cycles of vaccination based on ASEL recombinant oncolytic viruses without triggering autoimmunity. The success of this tedious enterprise relied upon several factors: (1) intravenous inoculation of the ASEL (as opposed to local intraprostatic inoculations which provoked prostatitis), (2) mounting protective $CD4^+TH17$ systemic immune responses against tissue-specific antigens (as opposed to $CD8^+$ Tc1 cells), (3) presentation of a broad repertoire of low affinity antigens (as opposed to immunodominant ones), (4) the use of xenogeneic, altered self antigens (of human as opposed to mouse origin).[3]

The same highly immunogenic virus was subsequently utilized to screen for tumor rejection antigens (TRA) as opposed to tumor-associated antigens (TAA) in a syngeneic context, in B16F10 mouse melanoma. Interestingly, only three TRA (TRP1, NRAS, cytochrome C1) that triggered a tumor/tissue-specific $CD4^+TH17$ response in vitro, could be identified in the whole cDNA library. When used in combination in a tripartite vaccine (but not one by one), the three TRA-expressing VSV clones cooperated to induce potent antitumor immune responses leading to tumor rejection (in the prophylaxis, as well as the therapy of B16F10 melanoma), achieving the same efficacy as the unselected parental virus library.[3] These studies revealed that altered self-antigens that are administered in a viral context can overcome immunological tolerance to cancer antigens. Interestingly, both NRAS and cytochrome C1 cDNAs from B16 melanomas contained point mutations, although the contribution of these mutations to the immunogenicity of the viral vaccine remained elusive.

The question as to whether mutations confer immunogenicity has been addressed by Castle et al. in the same B16F10 tumor model.[4] From a classical immunological perspective, mutated antigens may be particularly useful in therapeutic tumor vaccines because mutations create neoantigens against which no complete immunological tolerance has been induced, for instance by deletion of the self-reactive T-cell repertoire in the thymus. By analyzing the first mouse tumor exome, Castle et al. identified 962 non-synonymous somatic point mutations, 563 of which occurred in genes that were actually expressed by B16F10 cells.[4] None of these mutations resided in the four melanoma oncogenes. In contrast, three melanoma-relevant tumor suppressors displayed mutations. More importantly, mutations were discovered in crucial signaling pathways (such as RAS/MAPK/ERK and PIEK/AKT), in the Trrap gene (shared between mouse and human melanoma), in the DNA repair machinery and in several genes of general relevance to oncogenesis (such as Alk, Aim1, Flt1 and Fat1). At the next step, the authors performed a systematic screening to measure the immunogenicity of a selected panel of proteins (affecting ~50 proteins). For this purpose, naïve C57BL/6 mice were vaccinated with long synthetic peptides corresponding to the mutant poteins in adjuvant

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Submitted: 04/21/12; Accepted: 04/23/12
http://dx.doi.org/10.4161/onci.20730

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[Poly (I:C)]. Then the recall response of splenocytes to RNA (encoding the mutated antigen)-transfected dendritic cells was evaluated by ELISPOT assays. One third of the mutated epitopes were strongly immunogenic (and hence as efficient as the positive control Trp2). Moreover, roughly half of this third of immunogenic peptides, cross-elicited T-cell responses against the non mutated peptide. Importantly, no apparent correlation between the immunogenicity and the oncogenicity of the target molecule could be uncovered, meaning that "driver" and "bystander" mutations do not differ in their immunological outcome.[4]

The question as to whether nascent tumor cells express neoantigens that could contribute to their T-cell-dependent editing in immunocompetent hosts has been recently adressed by Schreiber and coworkers.[5] Unedited methylcholanthrene-induced sarcomas (that are induced in immunodeficient mice and hence have not been edited by the immune system) induce potent immune responses when implanted into immunocompetent hosts. The immunogenicity of these tumors can be explained by the expression of highly antigenic mutant proteins such as mutant spectrin-β2. T lymphocytes raised against the mutant spectrin-β2 eliminate tumor cells expressing the mutant antigen and indirectly promote the outgrowth ("immunoselection") of cells lacking this antigen.[5] The immunodominance of mutant spectrin-β2 in driving tumor rejection might be explained by the presence of four copies of chromosome 11 in the immunogenic tumor variant. Each copy carries the mutant spectrin- β2 gene which produces abundant amounts of neoepitopes binding to H2-D^b with a 750-fold higher avidity than the non--mutated version of the epitope. Interestingly, the immunoepitope analysis of the parental sarcoma line that never gave rise to escape variants revealed a more complex array of neoantigens (19 strong binders to H-2D^b and 58 to H2-K^b) capable of inducing efficient MHC Class I-restricted $CD8^+$ T cell responses in vivo, suggesting the potency of TRA cooperation.

One could argue that carcinogen-driven tumors might be more immunogenic than oncogene-driven, endogenous cancers.[6,7] However, Tyler Jacks and coworkers provided evidence that Kras/p53-driven sarcoma engineered to express a TRA (namely luciferase) as a tumor-neoantigen have the potential to become immunoedited in a $CD4^+$ and $CD8^+$ T cell-dependent fashion by loosing the TRA or MHC Class I molecules.[8] They elegantly reversed the immunoselection pathway (driven by epigenetic silencing of the neoantigen expression) using DNA methyltransferase inhibitors (5 aza-2'-deoxycytidine). Their findings also suggest that tumor immunogenicity is not a universal characteristic of cancer development. Indeed, Kras/p53-driven sarcomas devoid of genetically driven TRA expression led to reduced intrinsic immunogenicity despite of the absence of previous exposure to adaptive immunity.[8]

The possibility to use modest amounts of DNA as a starting material and the ever-increasing cost and time efficiency of the next generation sequencing technology will facilitate genome-wide discovery of cancer-specific mutations and individualized cancer vaccines for patients.[9] Interestingly, in advanced disease where tumor genomes become more unstable and where tumor heterogeneity prevails,[10] single cell exome sequencing[11] may allow to tackle the potentially immunogenic mutanome more easily than other treatment options.[12] These efforts may revolutionize the prioritization list of cancer antigens than has ben assembled based on the available data.[13]

References

1. Segal NH, Parsons DW, Peggs KS, Velculescu V, Kinzler KW, Vogelstein B, et al. Epitope landscape in breast and colorectal cancer. Cancer Res 2008; 68:889-92; PMID:18245491; http://dx.doi.org/10.1158/0008-5472.CAN-07-3095
2. Kottke T, Errington F, Pulido J, Galivo F, Thompson J, Wongthida P, et al. Broad antigenic coverage induced by vaccination with virus-based cDNA libraries cures established tumors. Nat Med 2011; 17:854-9; PMID:21685898; http://dx.doi.org/10.1038/nm.2390
3. Pulido J, Kottke T, Thompson J, Galivo F, Wongthida P, Diaz RM, et al. Using virally expressed melanoma cDNA libraries to identify tumor-associated antigens that cure melanoma. Nat Biotechnol 2012; 30:337-43; PMID:22426030; http://dx.doi.org/10.1038/nbt.2157
4. Castle JC, Kreiter S, Diekmann J, Löwer M, van de Roemer N, de Graaf J, et al. Exploiting the mutanome for tumor vaccination. Cancer Res 2012; 72:1081-91; PMID:22237626; http://dx.doi.org/10.1158/0008-5472.CAN-11-3722
5. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 2012; 482:400-4; PMID:22318521; http://dx.doi.org/10.1038/nature10755
6. Lee W, Jiang Z, Liu J, Haverty PM, Guan Y, Stinson J, et al. The mutation spectrum revealed by paired genome sequences from a lung cancer patient. Nature 2010; 465:473-7; PMID:20505728; http://dx.doi.org/10.1038/nature09004
7. Pleasance ED, Stephens PJ, O'Meara S, McBride DJ, Meynert A, Jones D, et al. A small-cell lung cancer genome with complex signatures of tobacco exposure. Nature 2010; 463:184-90; PMID:20016488; http://dx.doi.org/10.1038/nature08629
8. DuPage M, Mazumdar C, Schmidt LM, Cheung AF, Jacks T. Expression of tumour-specific antigens underlies cancer immunoediting. Nature 2012; 482:405-9; PMID:22318517; http://dx.doi.org/10.1038/nature10803
9. Kozarewa I, Rosa-Rosa JM, Wardell CP, Walker BA, Fenwick K, Assiotis I, et al. A modified method for whole exome resequencing from minimal amounts of starting DNA. PLoS One 2012; 7:e32617; PMID:22403682; http://dx.doi.org/10.1371/journal.pone.0032617
10. Roychowdhury S, Iyer MK, Robinson DR, Lonigro RJ, Wu YM, et al. Personalized oncology through integrative high-throughput sequencing: a pilot study. Sci Transl Med 2011; 3:111ra121
11. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012; 366:883-92; PMID:22397650; http://dx.doi.org/10.1056/NEJMoa1113205
12. Xu X, Hou Y, Yin X, Bao L, Tang A, Song L, et al. Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor. Cell 2012; 148:886-95; PMID:22385958; http://dx.doi.org/10.1016/j.cell.2012.02.025
13. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res 2009; 15:5323-37; PMID:19723653; http://dx.doi.org/10.1158/1078-0432.CCR-09-0737

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