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EDITORIAL

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A bacterium-derived, cancer-associated immunopeptidome

The immune system is endowed with the capacity to combat alien invaders including bacterial species and to eliminate endogenous deviants such as transformed cells. In this context, much attention has been paid to the possibility that bacterial and tumor antigens may be cross-reactive, explaining how the composition of the microbiota influences cancer immunosurveillance[1–3]. In an interesting twist, a recent *Nature* paper by Kalaora et al.[4] describes the identification of intracellular bacteria that alter the antigenic characteristics of human melanomas, thus potentially facilitating their immune recognition.

Successful anticancer immunotherapy relies on the capacity of the immune system to selectively recognize and destroy malignant cells, though without harming essential normal cell types.[5] For this reason, tumor immunologists attempt to identify tumor antigens that are exclusively expressed by cancer cells, not by their normal counterparts. One unfolding strategy for identifying cancer-specific antigens combines genomic or whole-exome sequencing with algorithms to identify mutations in genes/proteins that yield novel MHC class-I or class-II binding peptides (so-called neo-antigens) that can be presented by malignant or dendritic cells in the tumor microenvironment to T lymphocytes. This computational strategy can be complemented by methods in which peptides eluted from MHC molecules are subjected to mass spectrometry (MS). Knowing the exact molecular mass of the peptides, it is possible to predict their sequence among a range of possible peptides encoded by the cancer cell genome. This is an important and critical point, meaning that the reference catalog of possible peptides strongly influences the results of their MS identification. Thus, when the exome (i.e. the sum of all predicted protein-coding regions in the genome) is used as a reference catalog, logically only peptides from the canonical proteome can be identified within the immunopeptidome. However, if all possible RNA-derived peptides (including peptides encoded from introns and so-called untranslated regions and non-coding RNAs) are included in the reference catalog, evidence emerges that a large portion of the immunopeptidome is not derived from the canonical proteome and rather corresponds to RNAs that are translated in an abortive, nonproductive fashion) .[6,7]

Kalaora et al.[4] used deep sequencing to identify small amounts of bacterial DNA in melanomas (which were comparable to the levels of bacterial DNA in peripheral blood from the same patients), allowing to identify the corresponding bacterial species and to validate by in situ hybridization that a fraction of the tumor cells indeed contained bacterial DNA. Kalaora et al.[4] then performed mass spectrometric immunopeptidome analyses using as the reference catalog the human proteome (but only the canonical, exome-based proteome) and the proteome of the bacterial species identified by the genomic analyses, while filtering the data based on the predicted ability of the peptides to bind the HLA alleles of the patient. This procedure led to the identification of multiple bacterium-derived peptides, some of which were shared among different patient samples, although most of the peptides are "private" in the sense that they were only found in one among the 17 melanoma metastases from 9 patients.

While bacterial antigens represent an unexpected source of peptides presented by HLA molecules of melanoma patients, other considerations are worth taking into account to comprehensively search large-scale proteome datasets. First, peptides from non-canonical human proteins, representing about 10% of the HLA class I immunopeptidome, [8] have not been taken into account in the study by Kalaora et al. .[4] Secondly, putative sequences can be assigned from an incomplete set of fragment ions, and inverted amino acids could provide meaningful alternative antigen sequences as some isomeric peptides could co-elute and be difficult to distinguish from one another (e.g. LSDLGK**SI**Y attributed to *Staphylococcus aureus* potentially matches LSDLGK**LS**Y from human histone acetyltransferase KAT8). Third, some peptides could be shared between bacterial species or could be difficult to identify if whole genome sequencing is not available. For example, ALGVDALLLL and ITDFIDPNQY (attributed to *S. aureus* by Kalaora et al.[4]) could not be found in the Uniprot databank, but rather may be derived from *Staphylococcus schweitzeri* (from the source genes opuCB and bglA, respectively), which is not yet known as a pathogen for humans. Similarly, doubts can be cast on the identification of a plant pathogen such as *Sphingomonas melonis* (known to cause brown spots on melon fruits) in human melanoma specimens.

Irrespective of these caveats, the work by Kalaora et al.[4] raises the possibility that a minor fraction of human melanoma cells and melanoma-associated immune cells contain live bacteria that contribute specific epitopes to the immunopeptidome. This work is an eloquent demonstration of an essential and frequently overlooked principle: mass spectrometrists can only identify peptides represented in their proteomic search space. Hence, one could never identify bacterial peptides unless the bacterial proteome is purposely included in the reference database of MS analyses. *Fusobacterium nucleatum*, a bacterium endowed with oncogenic and immunosuppressive activity, for instance in colorectal cancer, [9,10] contributed several epitopes in two distinct patients. Two other common pathogens, *S. aureus* and *S. capitis* also contributed several epitopes, but multiple other intracellular and extracellular bacteria may generate MHC class-I or class-II binding peptides as well .[4] It this finding is confirmed, it will be interesting to understand whether the low frequency of intracellular bacteria found in melanoma results from immunosurveillance by bacterial epitope-specific T cells that eradicate those tumor cells

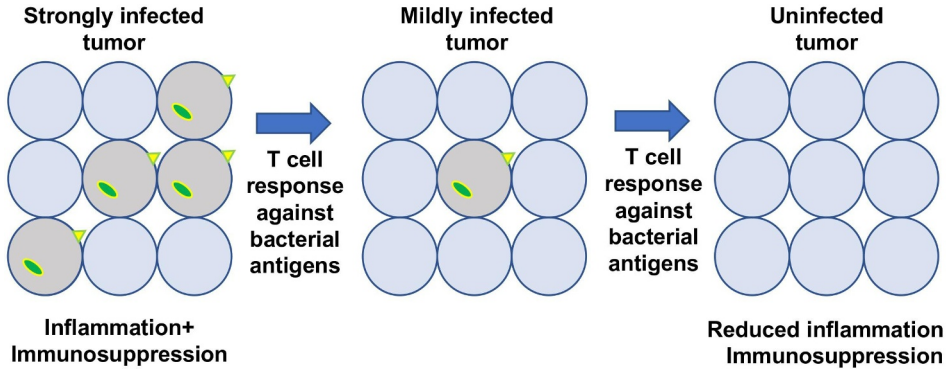

Figure 1. Hypothetical relationship between intracellular bacteria, the expression of MHC class-I- restricted epitopes on the surface of infected tumor cells and immunosurveillance by cytotoxic T lymphocytes eliminating infected tumor cells. In the real-life situation, in patients with metastatic melanoma, a minor fraction of tumor cells contains bacteria (middle panel). It will be interesting to know whether local immunosuppression may shift the balance to a more intense bacterial infection or whether, on the contrary, improved T cell responses against bacterium-encoded epitopes (symbolized by triangles) can eradicate infected tumor cells.

that contain the relevant pathogen, yet spare non-infected cells (Fig.1). Moreover, the intriguing possibility emerges to artificially enhance such antibacterial immune responses, which then might favor epitope spreading, ultimately leading to the recognition and destruction of non-infected melanoma cells. Alternatively, the eradication of tumor cells containing pro-inflammatory bacteria might favorably remodel the tumor microenvironment to relieve local immunosuppression.

In light of these perspectives, future research should unveil the contribution of tumor-resident bacteria to the cancer-immune dialogue. To start with, it will be important to systematically explore the possibility that cancer-associated immunopeptidomes contain epitopes derived from the microbiome.

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GK is a co-founder of everImmune.

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Claude Perreault and Pierre Thibault
aInstitute for Research in Immunology and Cancer, Université De Montréal, Montréal, Canada

Guido Kroemer
bKarolinska Institute, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden
cMetabolomics and Cell Biology Platforms, Institut Gustave Roussy, Villejuif, France
dEquipe Labellisée Par La Ligue Contre Le Cancer, Université De Paris, Sorbonne Université, Inserm U1138, Centre De Recherche Des Cordeliers, Institut Universitaire De France, Paris, France
ePôle De Biologie, Hôpital Européen Georges Pompidou, Paris, France
fSuzhou Institute for Systems Medicine, Chinese Academy of Medical Sciences, Suzhou, China,CONTACT Guido Kroemer kroemer@orange.fr Centre de Recherche des Cordeliers, 14 rue de l'Ecole de Médecine, 75006 Paris, France
http://orcid.org/0000-0002-9334-4405

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