Epigenetically controlled tumor antigens derived from splice junctions between exons and transposable elements

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一、簡述論文的概要與重大發現

設若去除抑制轉座子(Transposable element, TE)中、對其選擇性剪接(alternative splicing) 之表觀遺傳性修飾、亦或相應序列之缺失，咸認會造成外顯子(exon) 和轉座子之間的非典型剪接，而其產物: exon-TE splicing junction, JET 會進而影響腫瘤特異性抗原的表達。因此，作者想要探討此非典型剪接位點所產生的產物能否作為腫瘤特異性抗原的來源。

作者首先比較來自小鼠 MC38、B16、MCA101 腫瘤細胞及正常組織所表達的 JET，發現其中一些是具有腫瘤特異性的。接著在腫瘤細胞中透過 immunopeptidome analyses 發現 JET 可以編碼出含 MHC-I (第一類主要組織相容性複合體)之胜肽，且其在具腫瘤的小鼠中是具有免疫抗原性的(immunogenic)，並且使用衍生自 JET 的胜肽進行免疫治療發現其可以有效減緩腫瘤的生長。最後作者將組蛋白甲基轉移酶 (histone methyltransferase) Setdb1 失活，以去掉對 TE 轉座的抑制，結果發現它會進一步影響 JET 的表達，並且能夠提高腫瘤免疫抗原性 (tumor immunogenicity)。

二、對論文內容的疑問

此篇研究的樣本都來自相同基因的 C57BL6/J 小鼠，但不同個體間的基因可能存在差異，因此如果去比較不同個體間 JET 的表達是否會得到不同的結果? 在正常細胞內即存在 JET，這些 JET 是否仍具生理功能應先探討。

三、論文的缺點與評論

此篇研究中發現在小鼠中，JET 會受到表觀遺傳性修飾的控制，並且產生各種免疫抗原性腫瘤抗原和保護性腫瘤抗原，這些發現或為癌症患者的腫瘤標靶疫苗的開發提供了新的思路。

CANCER IMMUNOLOGY

Epigenetically controlled tumor antigens derived from splice junctions between exons and transposable elements

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Oncogenesis often implicates epigenetic alterations, including derepression of transposable elements (TEs) and defects in alternative splicing. Here, we explore the possibility that noncanonical splice junctions between exons and TEs represent a source of tumor-specific antigens. We show that mouse normal tissues and tumor cell lines express wide but distinct ranges of mRNA junctions between exons and TEs, some of which are tumor specific. Immunopeptidome analyses in tumor cell lines identified peptides derived from exon-TE splicing junctions associated to MHC-I molecules. Exon-TE junction–derived peptides were immunogenic in tumor-bearing mice. Both prophylactic and therapeutic vaccinations with junction-derived peptides delayed tumor growth in vivo. Inactivation of the TE-silencing histone 3–lysine 9 methyltransferase *Setdb1* caused overexpression of new immunogenic junctions in tumor cells. Our results identify exon-TE splicing junctions as epigenetically controlled, immunogenic, and protective tumor antigens in mice, opening possibilities for tumor targeting and vaccination in patients with cancer.

INTRODUCTION

With the therapeutic successes of immune checkpoint inhibitors (*1*), it has become clear that the immune system can target tumors and induce their effective rejection through T cell responses in patients with cancer. Tumor rejection by the immune system occurs through recognition of tissue differentiation and tumor/testis antigens presented by major histocompatibility complex (MHC) molecules on tumor cells. A different family of tumor antigens derives from passenger or driver mutations present in clonally expanded tumor cells (*2*). Some of these mutations enhance the affinity of peptides for MHC molecules and thus become bona fide tumor-specific antigens (*3*, *4*). The frequency of random tumor mutations, however, may not account for all antitumor T cell responses in patients with cancer. Many patients bearing cancer types with low mutation burdens respond to checkpoint blockers, and the correlation between the number of mutations and the response to checkpoint inhibitors is weak at the individual patient level (*5*, *6*). Recent studies have investigated the possibility that transcripts from noncoding genomic regions may be a source of shared, although rare, tumor antigens (*7*–*11*).

Transposable elements (TEs) represent between 40 and 50% of mammalian genomes and originate from sequences capable of transposing, i.e., replicating and inserting in distal parts of the genome (*12*). De novo insertions from full TE transposition cycles can threaten genome stability. Most TE-derived sequences in mice are partially degenerated and have lost the capacity to transpose. However, they can regulate gene expression, acting as promoters or enhancers or providing alternative translation start sites (*13*). TEs can also provide alternative splice sites (often referred to as "noncanonical") (*14*), but the contribution of these splicing events to tumor immunogenicity has not been investigated so far. As a protection mechanism, mammalian cells have developed epigenetic mechanisms to repress TE expression and transposition, including DNA methylation and various histone modifications (*15*, *16*). These mechanisms can be compromised in tumor cells, leading to a partial release of TE transcriptional control (*17*). In this study, we explore the possibility that epigenetic defects in tumor cells, including derepression of TEs, generate tumor-specific antigens derived from noncanonical splicing events involving TEs.

RESULTS

TE-exon junction identification pipeline

To investigate whether TEs alternatively splice with exons, we developed a pipeline identifying nonannotated RNA sequencing (RNA-seq) split reads (*18*) bearing junctions involving a TE and a coding exon (Fig. 1A, further referred to as "JETs"). We term "donor" the 5′ part, "acceptor" the 3′ part, and "breakpoint" the position where

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