Original research

Epigenetic promoter alterations in GI tumour immune-editing and resistance to immune checkpoint inhibition

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一、簡訴論文概要及重大發現

本論文透過生物資訊析的方式，利用 ChIP-seq 找出在癌症/正常病人不同基因的 promoter 上 H3K4Me3 的變化(增加/減少)，再把 promoter 分成 gain promoters 或 loss promoters，結合 RNA-seq 的結果，利用作者的演算法「proActiv」，將 sample 分為 APB high/int/low，並針對三種分類進行實驗。

作者的目的是探討 APB 與免疫編輯、免疫療法抗性的關係，所以作者首先利用 TCGA 的胃癌病人 sample，測量 APB high/int/low 三組中 CD8A，GZMA 及 PRF1 這些 T-cell cytolytic markers 的表達量，接著再測量 9 個與 immune checkpoint 相關的基因表達量。結果都可以發現，在 APB low 的情況下有高的 T-cell cytolytic markers 表達及 immune checkpoint 基因表達。

接著作者對病人的癌組織 sample 做同樣的 APB 分類以及測量 immune checkpoint 相關的基因表達量，與 TCGA 的結果一樣，APB low 的情況下有高的表達。

然後作者再利用 humanized mice 以及 NSG 免疫缺憾鼠作對照，把對應 APB high/int/low 的三種癌細胞打到肝內。一個月後犧牲老鼠看癌腫的狀況，發現在 APB high 的老鼠中，humanized mice 的腫瘤比 NSG 老鼠大，而 APB low 的老鼠，humanized mice 的腫瘤比 NSG 老鼠小。同時在 APB low 的老鼠 IHC 中，CD8/CD3 的量也比 APB high 的老鼠高，說明在 APB low 的老鼠有 T-cell infiltration。

作者接下來利用經過 ICI 治療的病人 sample 作分析，把他們分類成 APB high/int/low 以後，看他們 promoter isoform 的狀態，可以發現在 APB high 中有更多的 gain/loss promoter，並且在 APB high 的病人中存活率比 APB low 的病人低。

最後作者嘗試跳出胃癌，透過 TCGA 針對 26 種不同癌症作相同的 APB high/int/low 分類，結果顯示除了 3 種癌症以外，其他 23 種都與胃癌一樣，在 T-cell cytolytic markers 中 APB low 具有較高的表達量。

總結上述的結果，APB 可以在不同的癌症免疫治療中作為 negative predictive biomarker。

二、對論文內容的提問

既然作者的目的是探討 APB 與免疫編輯、免疫療法抗性的關係，為什麼在小鼠實驗中沒有針對老鼠施打 ICI 藥物然後看牠們的免疫相關基因表達為何？

三、論文的缺點與評論

作者利用了新的演算法把 sample 分成 APB high/int/low，並發現了它們可以當作 negative predictive biomarker。但我覺得作者可以在老鼠實驗的部份多做一點實驗，例如看牠們的 promoter isoform 的狀態，還有使用 ICI 藥物看能不能治療癌症。會讓實驗更有說服力。

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ABSTRACT

Objectives Epigenomic alterations in cancer interact with the immune microenvironment to dictate tumour evolution and therapeutic response. We aimed to study the regulation of the tumour immune microenvironment through epigenetic alternate promoter use in gastric cancer and to expand our findings to other gastrointestinal tumours.

Design Alternate promoter burden (APB) was quantified using a novel bioinformatic algorithm (*proActiv*) to infer promoter activity from short-read RNA sequencing and samples categorised into APB_{high}, APB_{int} and APB_{low}. Single-cell RNA sequencing was performed to analyse the intratumour immune microenvironment. A humanised mouse cancer in vivo model was used to explore dynamic temporal interactions between tumour kinetics, alternate promoter usage and the human immune system. Multiple cohorts of gastrointestinal tumours treated with immunotherapy were assessed for correlation between APB and treatment outcomes.

Results APB_{high} gastric cancer tumours expressed decreased levels of T-cell cytolytic activity and exhibited signatures of immune depletion. Single-cell RNAsequencing analysis confirmed distinct immunological populations and lower T-cell proportions in APB_{high} tumours. Functional in vivo studies using 'humanised mice' harbouring an active human immune system revealed distinct temporal relationships between APB and tumour growth, with APB_{high} tumours having almost no human T-cell infiltration. Analysis of immunotherapy-treated patients with GI cancer confirmed resistance of APB_{high} tumours to immune checkpoint inhibition. APB_{high} gastric cancer exhibited significantly poorer progression-free survival compared with APB_{low} (median 55 days vs 121 days, HR 0.40, 95% CI 0.18 to 0.93, p=0.032).

Conclusion These findings demonstrate an association between alternate promoter use and the tumour microenvironment, leading to immune evasion and immunotherapy resistance.

Significance of this study

What is already known on this subject?

- Immune escape is a key factor for tumourigenesis.
- Epigenetic alterations in cancer interact with the immune microenvironment to control tumourigenesis and response to therapy.
- Studies in gastric cancer have shown an association between epigenetic alternate promoters, immune-editing and immune checkpoint inhibitor (ICI) resistance.

What are the new findings?

- Gastric tumours with higher epigenetic promoter alterations exhibited decreased levels of T-cell cytolytic markers and expressed signatures of immune depletion.
- These findings were orthogonally validated using novel technologies and platforms such as single-cell RNA sequencing and 'humanised mice'.
- Multiple gastrointestinal tumour types with higher alternate promoter burden also correlated significantly with poorer survival with ICI therapy.

How might it impact on clinical practice in the foreseeable future?

- Alternative promoter use burden may represent a negative predictive biomarker for immunotherapy applicable to multiple gastrointestinal tumour types.

INTRODUCTION

Tumour growth and metastases in the presence of robust host immunosurveillance is a hallmark of cancer. Immune-editing is a process harnessed by tumour cells to evade immune recognition using mechanisms such as modifications in antigen