Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer

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(1) 簡述論文的重大發現

在腸道中，腫瘤與相關微生物之間得關係時常被研究，以往研究因技術限制只能透過萃取大量組織細胞來進行分析，而導致無法觀察微生物在腫瘤的分佈及表現，但現今空間分析技術的進步，單細胞定序技術，此篇文章對口腔鱗狀細胞和結直腸癌細胞觀察微生物與癌細胞之間的空間、細胞和分子的分佈。

研究團隊透過 10x Visium 技術來定位患者組織中微生物在癌細胞中的種類以及分佈位置，發現細菌在癌症中並不是平均分佈，而是會集中在某一處，透 過RNAscope原位雜交定量分析技術，觀察到細菌對癌細胞蛋白質表現影響， 上皮細胞區域 Ki-67 的表達下降，免疫細胞區域 CTLA4 及 ARG1 等上升，表現出是區域化的影響，另外作者研發出 INVADEseq (invasion–adhesion-directed expression sequencing) 單細胞 RNA 定序技術，用來了解腫瘤微環境中微生物對細胞產生異質化的作用，最後作者在微生物對癌症細胞的遷移影 響中，觀察到在具核梭桿菌 (*F.nucleatum*)具有增強單細胞的遷移能力。

(2) 對論文內容的疑問

文中有提到受到具核梭桿菌感染後細胞，增值標記 Ki-67 有被下調，照理會影響癌細胞的增殖，因此可以在這方面進一步實驗觀察微生物感癌細胞後對增值的影響。

(3) 論文的缺點與評論

這篇研究十分詳細且新穎，使用新開發的實驗技術及INVADEseq 單細胞定序，觀察在過去因技術受限而無法觀察到微生物在腫瘤內的分佈及作用， 現今對腫瘤與微生物之間關係有更進一步了解，未來會加上微生物的評估分析， 來進一步確認癌症患者用於預防或治療的標靶或藥物治療。

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The tumour-associated microbiota is an intrinsic component of the tumour microenvironment across human cancer types[1,2]. Intratumoral host–microbiota studies have so far largely relied on bulk tissue analysis[1–3], which obscures the spatial distribution and localized effect of the microbiota within tumours. Here, by applying in situ spatial-profiling technologies[4] and single-cell RNA sequencing[5] to oral squamous cell carcinoma and colorectal cancer, we reveal spatial, cellular and molecular host–microbe interactions. We adapted 10x Visium spatial transcriptomics to determine the identity and in situ location of intratumoral microbial communities within patient tissues. Using GeoMx digital spatial profiling[6], we show that bacterial communities populate microniches that are less vascularized, highly immuno-suppressive and associated with malignant cells with lower levels of Ki-67 as compared to bacteria-negative tumour regions. We developed a single-cell RNA-sequencing method that we name INVADEseq (invasion–adhesion-directed expression sequencing) and, by applying this to patient tumours, identify cell-associated bacteria and the host cells with which they interact, as well as uncovering alterations in transcriptional pathways that are involved in inflammation, metastasis, cell dormancy and DNA repair. Through functional studies, we show that cancer cells that are infected with bacteria invade their surrounding environment as single cells and recruit myeloid cells to bacterial regions. Collectively, our data reveal that the distribution of the microbiota within a tumour is not random; instead, it is highly organized in microniches with immune and epithelial cell functions that promote cancer progression.

In the tumours of patients with cancer, malignant cells are surrounded by a complex network of non-malignant cells that may have pro- or anti-tumorigenic effects depending on their cell type and abundance. In vitro and preclinical animal models indicate that bacteria in the tumour-associated microbiota have a role in cancer development[7], metastasis[8–10], immunosurveillance[11–13] and chemoresistance[14,15]. There is strong molecular evidence of an intratumoral microbiota across at least 33 major cancer types[2,12,13,16], as well as imaging data that show the co-localization of pan-bacterial markers with immune and epithelial cell targets, suggesting that the intratumoral microbiota can be intracellular[2,8,13]. However, the precise identity of these cell-associated organisms and the specific host cell types with which they interact in patient tumours have yet to be fully revealed. In addition, whether the spatial distribution of the intratumoral microbiota and specific host–microbial cellular interactions affect distinct functional capabilities within the tumour microenvironment (TME) is largely unknown. Here, focusing on cancers at the extremes of the gastrointestinal tract—oral squamous cell carcinoma (OSCC) and colorectal cancer (CRC)—we modify in situ spatial-profiling technologies and single-cell RNA sequencing (scRNA-seq) to concurrently map host–bacterial spatial, cellular and molecular interactions within the TME. Our results reveal how the intratumoral microbiota contributes to tumour heterogeneity.

Heterogeneity of the intratumoral microbiota

We performed 16S rRNA gene sequencing on 44 pieces of tissue from the tumours of 11 patients with CRC (Extended Data Fig. 1a), and observed that the composition of the intratumoral microbiota at the phylum and the genus level (Extended Data Fig. 1a and Supplementary Table 2), including *Fusobacterium* (Extended Data Fig. 1b), varied within individual patient tumours. Principal component analysis with beta diversity clustering (Extended Data Fig. 1c) and dendrogram analysis (Extended Data Fig. 1d) showed that over one third of the patients assessed ($n = 4$ out of 11) had relatively stable microbiome compositions; however, most patients ($n = 7$ out of 11) exhibited varying levels of heterogeneity in the intratumoral microbiome. This suggests a

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