Promoter-level transcriptome identifies stemness associated with relatively high proliferation in pancreatic cancer cells(Abstract_要旨)

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論文題目	Promoter-level transcriptome identifies stemness associated with relatively high proliferation in pancreatic cancer cells （高度増殖性を示す膵臓癌細胞が持つ幹細胞特性のトランスクリプトーム解析による同定）		

（論文内容の要旨）

Pancreatic cancer remains among the most lethal malignancies worldwide, and the incidence of this disease has been increasing slowly in recent years. The assessments of molecular markers and tumor progression signatures are essential contributors to treatment decisions, as a timely diagnosis could prevent 12–13% of patients in a precursor stage or with clinically unapparent disease from progressing toward end-stage disease. To date, three precursors of pancreatic cancer have been identified: pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN).Both pancreatic intraepithelial neoplasia (PanIN), a frequent precursor of pancreatic cancer, and intraductal papillary mucinous neoplasm (IPMN), a less common precursor, undergo several phases of molecular conversions and finally develop into highly malignant solid tumors with negative effects on the quality of life. This long-standing issue was approached by examining the following PanIN/IPMN cell lines derived from mouse models of pancreatic cancer: Ptf1a–Cre; $Kras^{G12D}$; $p53^{fl}$ and Ptf1a–Cre; $Kras^{G12D}$; $Brg1^{fl}$ PDAs, these murine PanIN and IPMN lesions can be used to generate transcriptome signatures representative of overall pancreatic cancer characteristics.

Advances in next-generation sequencing technologies such as cap analysis of gene expression (CAGE) have led to a comprehensive understanding of the regulatory processes applied to transcribed regions of the genome and the construction of an integrated overview of the transcriptome. Particularly, CAGE was originally used to construct a precise map of transcription start sites (TSSs) and elucidate the “promoteromes” of mammalian cells and tissues. The mRNA from these cells was subjected to a cap analysis of gene expression (CAGE) to map the transcription starting sites and quantify the expression of promoters across the genome. Two RNA samples extracted from three individual subcutaneous tumors generated by the transplantation of PanIN or IPMN cancer cell lines were used to generate libraries and Illumina Seq, with four RNA samples in total, to depict discrete transcriptional network between IPMN and PanIN, registered as GSE139648 in GEO. Moreover, in IPMN cells, the transcriptome presented to be enriched for suppressive and inhibitory biological processes. In IPMN cells, the upregulated promoters were enriched for the GO categories related to inhibitory enzymatic activity, negative regulation of cell communication and immune processes, indicating a relatively more suppressive signature when compared with that of PanIN cells. In contrast, the transcriptome of PanIN cells exhibited properties of stemness, which was demonstrated by the $Aldh^{+}$ system that PanIN cells contained a larger proportion of cells with stemness. Also, in a comparison of the cell lines, both the numbers of generated spheres with diameters >80 μm and the average area of a single sphere exhibited insignificant differences. After passage, however, PanIN exhibited a greater capacity in terms of overall sphere formation and spherical size. The varied sphere densities and diameters despite culture under the same circumstances revealed the diverse stemness properties of PanIN and IPMN cells. Notably, the proliferation capacity of the PanIN cells in culture was only minimally constrained by well-known chemotherapy drugs such as GSK690693 and gemcitabine. The various transcriptional factor network systems detected in PanIN and IPMN cells reflect the distinct molecular profiles of these cell types. In the future, it is hoped that these findings will enhance the mechanistic understanding of the characteristic molecular alterations underlying pancreatic cancer precursors. These data may provide a promising direction for therapeutic research.

（論文審査の結果の要旨）

膵臓癌は最も予後の悪い癌のひとつとされ、近年その頻度が増加傾向にあるため、病態の理解が求められている。ほとんどの膵臓癌は PanIN（pancreatic intraepithelial neoplasm）または IPMN (intraductal papillary mucinous neoplasm) を前癌病変として発生し、PanIN 由来と IPMN 由来で予後が異なることが知られている。そこで申請者は、遺伝子改変マウスから樹立した PanIN 由来膵臓癌、IPMN 由来膵臓癌の細胞株を用いて、両者の差異を検討した。

まず PanIN 由来膵臓癌、IPMN 由来膵臓癌の細胞株からゼノグラフトを作成後、RNA サンプルを抽出し、CAGE (cap analysis of gene expression)によってトランスクリプトーム・ネットワークの違いを検討した。その結果、PanIN 由来膵臓癌の細胞株は、幹細胞性に関わるプロパティが優位であった。一方、IPMN 由来膵臓癌の細胞株は、増殖抑制性のプロパティが優位な傾向にあった。さらに両者の培養細胞株を用いて薬剤投与実験を行ったところ、PanIN 由来膵臓癌の細胞株は AKT 阻害剤に抵抗性を示した。

本研究は、PanIN 由来膵臓癌と IPMN 由来膵臓癌の遺伝子発現と生物学的動態の差異の一端を明らかにし、膵臓癌に対する治療法に寄与する可能性がある。

したがって、本論文は博士（医科学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和 2 年 7 月 1 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降