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Project Title	Understanding of epigenetic heterogeneity in intractable cancers	
Principal Investigator	Patrick Tan (Duke-NUS Medical School・Professor)	
Project Member(s)	**IMSUT Host Researcher**	Tatsuhiro Shibata (The Institute of Medical Science, The University of Tokyo・Professor)
	Project Member (s)	Kohshi Mimori (Department of Surgery, Kyushu University Beppu Hospital・Professor)
	Project Member (s)	Yutaka Kondo (Division of Cancer Biology, Nagoya University Graduate School of Medicine・Professor)
	Project Member (s)	Tatsuhiro Shibata (Laboratory of Molecular Medicine・Professor)
	Project Member (s)	Atsushi Niida (Laboratory of Molecular Medicine・Senior Assistant Professor)
	Project Member (s)	Kazuki Takahashi (Laboratory of Molecular Medicine・Assistant Professor)

(Form 2-2)

Principal Investigator

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Joint Research Report (Annual/Project Completion)

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Report

【Decoding epidrivers of gastric cancer】
In the review period, we collaborated with the members of this collaborative research on a large-scale genomic analysis of gastric cancer (GC), pooling together >1000 GC cases from several countries, including Japan and Singapore. This work has revealed a host of new driver genetic alterations including chromatin modifying enzymes such as SWI/SNF complex, which may influence the chromatin landscape of gastric tumors. This work is currently in press at the Nature Genetics journal.

We are also working on a manuscript describing the different functional effects of ARID1A inactivation in GC. ARID1A, a chromatin modifier gene, is one of the most frequently mutated tumor-suppressor genes in GC and other tumor types; however, it is still being determined if the molecular pathways regulated by ARID1A in different tissues are similar or different. Our preliminary data suggest that ARID1A loss may influence changes in the tumor microenvironment that highlight new possibilities for targeting ARID1Amutated
GCs.

【Enhancer landscape of pancreatic cancer】
Pancreatic cancer has a poor prognosis, with a 5-year survival rate of about 10%. Although the significant driver mutations in pancreatic cancer are already known, they have not yet been identified as therapeutic targets. Therefore, we would like to find new therapeutic targets by focusing on enhancer regions that regulate gene expression. We used NET-CAGE as a new technology for enhancer analysis, in which enriched Nascent RNA (newly synthesized RNA from nuclear fractions) is subjected to CAGE. Compared to the conventional CAGE method, the detection sensitivity of activated enhancer RNA (eRNA) is dramatically improved. Samples implemented for analysis were pancreatic cancer cell lines PANC-1, MIA PaCa-2, PK-1, PK-59, PK-45H, PK-45P, PK-8, and T3M-4. NETCAGE analysis was implemented on HPDE-6/E6E7 and hTERTHPNE as normal pancreatic epithelial cells. When enhancer regions overlapped between cell lines, we defined them as the same enhancer and identified 28762 enhancer regions. In silico analysis was conducted to
narrow down the search further. We searched for enhancer site Z that fits the following conditions with novel target gene Y of transcription factor X, which is essential in pancreatic cancer: (1) Signal is present in NET-CAGE. (2) There is a signal in histone acetylation data of panc1 and other pancreatic cancer cell lines. (3) There is a motif of X. (4) There are ChIP-seq and binding signal data for X. In addition, we searched for Y in genes downstream of enhancer sites that fit the following conditions using TCGA: (1) Correlation with X by RNA-seq. (2) Elevated expression in RNA-seq tumors. (3) Significant pathway can be obtained when GSEA is applied to RNA-seq downstream gene candidates with continuous value sample labeling. (4) RNAi/CRISPR screening of pancreatic cancer cell lines has been reported in a previous report. We selected seven target transcription factors that fulfill the above criteria and genes that correlate with their expression. Currently, we are conducting experiments to narrow down the target genes further.

【Functional landscape of long non-coding RNAs in cancer】
To identify the long non-coding RNAs (lncRNAs) upregulated in response to Replication stress (RS) and Rloop accumulation, we used synchronized HeLa/Fucci2 cells. RS was induced by treatment with hydroxyurea (HU) or camptothecin (CPT) in the S phase, and we evaluated RS-induced lincRNA.

Among the upregulated lncRNAs, we identified the Small Nucleolar RNA Host Gene (SNHG) family, composed of more than 30 SNHGs (lncRNAs). Using the public database (TCGA), we found that some SNHGs are significantly upregulated
in gliomas and oral and pancreatic cancers. Currently, we further investigate the functional roles of the SNHGs during tumorigenesis.