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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Project Completion Report

Report

Decoding cancer chromatin landscape

We report an extensive, multi-ancestral landscape of driver events in gastric cancer, involving 1,335 cases.Seventy-seven significantly mutated genes (SMGs) were identified, including ARHGAP5 and TRIM49C. We also identified subtype-specific drivers, including PIGR and SOX9, which were enriched in the diffuse subtype of the disease. SMGs also varied according to Epstein-Barr virus infection status and ancestry. Non-protein truncating CDH1 mutations, which are characterized by in-frame splicing alterations, targeted localized extracellular domains and uniquely occurred in sporadic diffuse-type cases. In gastric cancer patients with East Asian ancestry, our data suggested a link between alcohol consumption or metabolism and the development of RHOA mutations. Moreover, mutations with potential roles in immune evasion were identified.

We examine 170 GC whole genomes to unravel the oncogenic structural aberration landscape in GC genomes and identify six rearrangement signatures (RSs). Non-random combinations of RSs elucidated unique GC subtypes comprising one or a few dominant RS that were associated with unique driver events (BRCA1/2 defects, mismatch repair deficiency, and TP53 mutation) and epidemiological backgrounds. Twenty-seven SV hotspots were identified as GC driver candidates. SV hotspots frequently constituted complexly clustered SVs involved in driver gene amplification, such as ERBB2, CCNE1, and FGFR2.

We have published a manuscript describing the different functional effects of ARID1A inactivation in GC (Xu et al., 2023 Gut). ARID1A, a chromatin modifier gene, is one of the most frequently mutated tumor-suppressor genes in GC and other tumor types, however it is unclear if the molecular pathways regulated by ARID1A in different tissues are similar or different. Our data suggests that ARID1A loss influences changes in the tumor microenvironment and rewires the GC epigenome highlighting new possibilities for combination targeting ARID1A-mutated GCs.

Cancer-specific enhancer landscape

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 hTERT-HPNE 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 genes of transcription factor X, which is essential in pancreatic cancer. 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. We compared the gene expression profile between Gene X plasmid transformed cell lines, PK45P, PK1 vector1, PK2 vector 2 and each parent cell, and validated the upregulated expression of target gene Y. In addition, we applied the single cell sequencing profile from the public database (Junya P. et al. Cell Research. 2019) to identify the origin of gene X derived cells, and found that gene X were derived from the ductal cell type1, which comprises the malignant pancreatic cells. Furthermore, we are analyzing the clinical significance of gene X and target gene Y expressions in another subgroup of 300 pancreatic cancer cases.

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 R-loop accumulation, we first synchronized HeLa/Fucci2 cells in the S phase and induced RS by treatment with hydroxyurea (HU) or camptothecin (CPT). Among the upregulated lncRNAs, we identified Small Nucleolar RNA Host Gene (SNHG) family, which are composed of more than 30 SNHGs (lncRNAs). Using the public database (TCGA), we found that some SNHGs are significantly upregulated in oral cancers, gliomas and pancreatic cancers as well. Among the SNHGs, SNHG7 is upregulated in many types of cancers including head and neck squamous cell carcinomas, and its elevated expression is correlated with a poor prognosis, as indicated by the analysis ofthe TCGA dataset.

SNHG7 is known to code two snoRNAs, which are a class of small RNA molecules that primarily reside in the nucleolus of eukaryotic cells. SnoRNAs are involved in the processing and modification of other RNA molecules, particularly ribosomal RNA (rRNA) and small nuclear RNA (snRNA). Suppression of SNHG7 in cancer cells downregulated the cell proliferation. Currently, we are focusing on understanding the specific role of SNHG7 in cancer formation. Investigating the functional domains or specific regions of SNHG7 can provide valuable insights into its molecular mechanisms and potential as a therapeutic target.

Development of This Project into the AMED-A*STAR Collaborative Project

During this project, our group applied for the AMED-A*STAR collaborative initiative (Tackling and Conquering Cancer Complexity) and successfully launched a new joint research project. This international collaboration has been highly fruitful and has played a significant role in fostering further international research partnerships.