学位論文抄録

Abstract of Thesis

Establishment and characterization of a novel cancer stem-like cell of cholangiocarcinoma

（胆管癌由来の新規癌幹細胞の樹立と性状解析）

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Background and Purpose: Cholangiocarcinoma (CCA) is aggressive and highly metastatic cancer with a poor response to chemotherapy and a high recurrence rate. Recent evidence suggests that cancer stem cells (CSCs) contribute to chemotherapy resistances and aggressive phenotype/recurrences of many cancers including CCA, and CSCs have been proposed as effective targets of cancer. However, the study of CCA-CSC is very limited since there are few CSC model cells and their molecular markers. In this study, a novel CCA-CSC model cell line has been established and characterized by the analyses of specific biological phenotypes, partial genomics/transcriptomis, and the specific molecular signalings by the deep global proteomics, to identify the specific target for the effective CCA treatment.

Methods: We established CCA-stem-like cells from CCA parental cell line, KKU-055-CSC under the specific condition of the stem cell medium after three months of successive sub-passages. The differentiation (DIF) of CSC is induced by the 10% FCS and observed phenotypic changes. The characterization of CSC properties compared with parental cells has been performed by genomic karyotype/STR analyses, mouse xenograft, qPCR/western blotting of stem cell markers, CK8 assay for cell proliferation and drug resistances, and multilineage differentiation assay by the specific mediums and stainings. The proteomics of those cells was performed by the label-free quantitation of Mass spectrometry DDA method using an Easy nanoLC-Orbitrap Fusion Tribrid system equipped with Nikkyo-RP-nano Column, The data mining; quantitative identification (FDR 1%), statistics, and cluster analyses, were performed by Proteome Discoverer (ver2.4, with Sequest), MaxQuant and Perseus software. Gene Ontology (GO) and network analyses were assisted by DAVID, KEGG, and KeyMolnet. In silico analysis, TCGA dataset in GEPIA online server was used. The cellular validations for extracted candidates were performed using the specific siRNAs and inhibitors.

Results: We successfully established a sphere-forming CCA stem-like cell, KKU-055-CSC, which grows stably and withstands continuous passage for a long period. Compared with its parental cancer cell line–KKU-055, the KKU-055-CSC noticeably exhibits the CSC characteristics, including 1) faster and constant expansive tumor formation in xenograft mouse models, 2) high expression of stem cell markers, 3) low responsiveness to standard chemo-drugs such as 5FU, cisplatin, gemcitabine, and 4) multi-lineage differentiation abilities to adipocyte and osteocyte in addition to CCA. Using These CCA-055-CSC, DIFF, and Parental cells, we have performed global proteomics to identify the CCA-CSC-associated pathway. Among 6000 quantitatively identified proteins in total, we extracted the protein groups upregulated in the CSC by cluster analysis. The network analysis revealed that High mobility group A1 (HMGA1) and Aurora-A signaling pathways via the STAT-3 pathways were enriched in CSC, besides the stem cell marker signaling upregulation, such as CD44, CD147, SOX2, EpCAM, Oct3/4, and ALDH. Knockdown of HMGA1 in KKU-055-CSC suppressed the expression of stem cell markers, induced the differentiation followed by cell proliferation, and enhanced sensitivity to chemo drugs including Aurora A inhibitors. *In silico* analysis demonstrated that the expression of HMGA1 was correlated with Aurora-A expressions and poor survival of CCA patients. These results suggest that the HMGA1 and Aurora-A signaling is essential pathways for CSC-CCA.

Conclusions: Our results demonstrated that KKU-055-CSC could be a useful tool for cancer research as a cancer stem cell model for CCA, and the HMGA1 and Aurora-A signaling pathway may be a novel candidate of therapeutic marker and target for CSC in cholangiocarcinoma.