[課程－２]

審査の結果の要旨

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This study was aimed i) to identify molecular markers to assess the gastric cancer cell fraction in any DNA sample, which will facilitate investigations of molecular mechanisms, and ii) to identify markers that can predict sensitivity of gastric cancers to DNA demethylating agents.

１． The establishment of a DNA methylation marker for the estimation of cancer cell fraction

1) Genome-wide DNA methylation analysis of 12 gastric cancer cell lines, 30 gastric cancer tissues, six normal gastric mucosae, one sample of peripheral leukocytes and four non-cancerous gastric mucosae identified three genomic regions (*OSR2*, *PPFIA3* and *VAV3*) that were barely methylated in normal cells, but were highly methylated in cancer cells.

2) qMSP validated that one or more of the three regions (*OSR2*, *PPFIA3* and *VAV3*) was highly methylated in all of the 26 gastric cancer tissues tested.

3) Analysis of four pairs of purified cells corroborated that the three genes (*OSR2*, *PPFIA3* and *VAV3*) were confirmed to be highly methylated (≥ 85 %) in cancer cells and barely methylated (≤ 5%) in non-cancer cells.

4) The cancer cell fraction assessed by the panel of the three genes (*OSR2*, *PPFIA3* and *VAV3*) showed good correlation with the fraction assessed by the *TP53* mutant allele frequency in 13 gastric cancers (r=0.77).

２． Identification of a sensitivity marker to 5-aza-2' -deoxycytidine (5-Aza-CdR)

1) Six and seven cell lines were found to be sensitive (IC50<0.04 μM) and resistant to 5-Aza-CdR (IC50>0.1 μM), respectively.

2) By comprehensive gene expression analysis, I identified a long noncoding RNA (*LINC00162*) that was highly expressed in sensitive cell lines (≥5/6).
3) Knock-down of *LINC00162* in NUGC3 and HSC41 cells decreased sensitivity to 5-Aza-CdR to 1/30 and 1/5, respectively. In addition, its overexpression in MKN74 and AGS cells increased sensitivity to 5-Aza-CdR to two- and three-fold, respectively.
4) Overexpression of *LINC00162* increased the sensitivity to 5-Aza-CdR in mouse xenograft model.

These data suggest that the new DNA methylation marker for cancer cell fractions will help the molecular characterization of gastric cancer, and that the new sensitivity marker, *LINC00162*, may be useful for the selection of gastric cancer patients to the treatment with 5-Aza-CdR. These findings are worth to deserving a degree conferral.