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学位論文題目	Alterations of Chromosomal Copy Number during Progression of Diffuse-type Gastric Carcinomas: Metaphase- and Array-based Comparative Genomic Hybridization Analyses of Multiple Samples in Individual Tumors （Diffuse-type 胃癌の進展における染色体 copy 数の変化：個々の腫瘍の複数サンプルの metaphase CGH と array CGH による解析）
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別紙様式３

論　文　内　容　要　旨

※整理番号	309	氏　名（ふりがな）	ポン　トン　ファ 彭　敦　發
学位論文題目	Alterations of Chromosomal Copy Number during Progression of Diffuse-type Gastric Carcinomas: Metaphase- and Array-based Comparative Genomic Hybridization Analyses of Multiple Samples in Individual Tumors (Diffuse-type 胃癌の進展における染色体 copy 数の変化：個々の腫瘍の複数サンプルの metaphase CGH と array CGH による解析)		

Purposes: *(1)* to detected DNA copy number imbalance in diffuse gastric cancers; *(2)* to discriminate earlier and later chromosomal changes during tumor progression and *(3)* to pick up putative target genes corresponding to the amplified regions detected by metaphase CGH.

Materials and Methods: A total of 69 tumor samples were taken from 19 cases with diffuse type gastric cancers, including 8 early cancers and 11 advanced cancers. In this study, we confined the examination to the tumors that had part of typical signet ring cell carcinoma in the mucosa and did not have any distinct tubular adenocarcinoma components. We used a laser capture microdissection system that enabled us to take about 100 to 2000 tumor cells from 2 to 8 different parts of individual tumors with the purity of 70 % or more. After extraction of DNA from microdissected cells, the tumor and normal reference DNAs were amplified by using a modified DOP-PCR protocol. This modified DOP-PCR protocol enabled us to get PCR products with the size more than 2 Kb up to 20 Kb, being suitable for subsequent nick translation labeling. For metaphase CGH, tumor DNA and reference DNA were labeled with FITC and TRITC respectively by nick translation. The labeled probes were mixed with human cot-1 DNA and co-hybridized to normal metaphase slides for 70 hours. Gains and losses were defined by green to red ratio (G/R) > 1.2 and < 0.8 respectively. Amplifications were defined by G/R ratio ≥ 1.5. Chromosomes 1p, 16p, 19, 22, and Y were excluded in the analyses.

For array CGH, tumor DNA and reference DNA were labeled with Cy3 and Cy5 respectively by random priming reaction. The labeled probes were mixed with human cot-1 DNA and co-hybridized to genomic DNA microarray (we used Vysis' GenoSensor array 300).

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等で印字すること。

２．※印の欄には記入しないこと。

The amplifications and losses were defined as T/R ratio > 2.0, and < 0.6 respectively. In order to assess clonal evolution process, we analyzed multiple samples from different parts of individual tumors by CGH, and compared the positions of breakpoints among the samples. We defined the common alterations shared by all the samples as stemline changes. The alterations that were not detected in all samples were defined as sideline changes.

Results: *(1)* The most frequent chromosomal aberrations detected by CGH were gains of 8q, 3q, 7q, 8p and loss of 17p, which were detected in more than 50% of the cases. *(2)* The gains of 8q, 8p, 1q and loss of 17p were picked up as frequent stem line changes. *(3)* By array CGH, *TERC/PIK3CA*, *MDM2*, *FES/IGF1R*, *FGFR2*, and *KRAS* were confirmed as the putative candidate target genes in the amplicons at 3q26.1-q27, 12q14-q15, 15q26, 10q24.3-q26.3, and 12p11.2-p12 respectively. And *TP53* and *CDH1* were confirmed to be involved in the frequent losses in 17p and 16q, respectively. *(4)* The frequencies of chromosomal aberrations, especially those stemline changes such as 8q+, 8p+, 7q+ and 17p-, were fairly similar between the samples of signet ring cell carcinomas and those of poorly differentiated carcinomas, whereas the gains of Xp, Xq, and 20q were more commonly detected in the samples of poorly differentiated carcinomas. *(5)* The frequencies of most chromosomal aberrations were similar between early cancers and advanced cancers, but the copy number losses of 3p, 18q, and copy number gains of 7p, 15q were more commonly detected in advanced cancers. *(6)* The samples with 7p+ also had greater number of changes than those without 7p+ ($p < 0.001$).

Discussions and Conclusions: *(1)* The cytogenetic changes detected by CGH remarkably varied from case to case, even in stemline changes, suggesting that the genetic pathways of gastric cancer may be quite diverse. *(2)* Array CGH is a useful screening method to list up putative target genes that corresponding to the amplified regions detected by CGH. *(3)* CGH analyses of multiple samples in individual tumors enabled us to discriminate earlier stemline changes (such as 8q+, 7p+ and 17p-) from later sideline changes (such as 11q-, 21q-, and 10q amplification). *(4)* Xp+, Xq+, 20q+were more commonly detected in the samples of poorly-differentiated cancers, suggesting that these aberrations may be important for the transition from signet ring cell carcinoma to poorly-differentiated carcinoma. *(5)* 7p+, 15q+, and 3p-, 18q-were more common detected in advanced cancers, suggesting that these alterations may associated with tumor progression from early stage to advanced stage. *(6)* 7p+ may also play a role in acceleration of chromosomal instability of gastric cancer.

別紙様式８

学位論文審査の結果の要旨

整理番号	309	氏名	彭　敦發

（学位論文審査の結果の要旨）

本研究は、comparative genomic hybridization (CGH)法を用いて、これまでCGHデータの少ないdiffuse-type胃癌に着目し、染色体copy数の変化を解析した。腺管成分のない典型的なdiffuse-typeの胃癌１９例を対象にして、laser capture microdissection法を用いて、一つの癌の多数箇所よりsampleを採取し、DOP-PCRによってDNAを増幅し、CGHを行った。一部のDNAはarray CGHに用いた。結果は以下に示す。

１）Diffuse type 胃癌では、8q+、17p-などの染色体コピー数の変化を多数検出した。

２）CGHでみられた12p、12q、10q、15qのamplicon に *KRAS*、*MDM2*、*FGFR2*、*FES* が含まれることをarray CGHで確認した。

３）8q+、8p+、1q+、17p-は早期のstemline に頻繁に観察された。

４）印環細胞癌から低分化腺癌への転換には、Xp+、Xq+、20q+などの変化が関係していると考えられた。

５）早期癌から進行癌への進展には7p+、15q+、3p-、18q-などの変化が関係しているらしい。

本研究は、世界で初めてCGHを使ってdiffuse-type胃癌の経時的な染色体copy数の変化を同定したものであり、この分野での貢献度は大きいといえる。従って、博士（医学）の学位を授与するに値するものと認められる。

（平成 15 年 9 月 1 日）