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学位の種類	博　士　（医　学）
学位記番号	博　士　甲第７３３号
学位授与の要件	学位規則第４条第１項該当
学位授与年月日	平成２７年　９月　９日
学位論文題目	Progression risk assessments of individual non-invasive gastric neoplasms by genomic copy-number profile and mucin phenotype. （ゲノムコピー数プロファイルと粘液形質による非浸潤性胃腫瘍の進展リスク評価）
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別紙様式３

論 文 内 容 要 旨

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学位論文題目	ゲノムコピー数プロファイルと粘液形質による非浸潤性胃腫瘍の進展リスク評価 Progression risk assessments of individual non-invasive gastric neoplasms by genomic copy-number profile and mucin phenotype		

Aims

Early detection and treatment of non-invasive neoplasms can effectively reduce the incidence of advanced gastric carcinoma (GC), but only when the lineage is continuous between non-invasive and advanced tumours. Although a fraction of non-invasive neoplasms progress to invasive GC, it is difficult to identify individual progression-prone non-invasive neoplasms. To classify non-invasive gland-forming gastric neoplasms into clusters of different levels of progression risk, we applied mucin phenotyping and genomic DNA microarray analyses to intramucosal gland-forming gastric neoplasms.

Methods

Formalin-fixed, paraffin-embedded tissues from 19 non-invasive and 24 invasive gland-forming neoplasms were obtained via endoscopic submucosal dissection or surgical excision. According to the Vienna classification, intramucosal neoplasms were classified as low-grade or high-grade non-invasive neoplasms (category 3, LGNs or group A and category 4, HGNs or group B, respectively) or invasive carcinomas (category 5 or group C). Group C was subdivided into Cm (intramucosal invasive carcinoma) and Cd (intramucosal part of submucosal or deeper invasive carcinoma). Neoplastic lesions were characterized by mucin phenotypes determined using monoclonal antibodies against MUC2, MUC5AC, MUC6, and CD10. Genomic DNA samples from mucosal neoplasms were subjected to array-based comparative genomic hybridization (aCGH) and subsequent unsupervised, hierarchical clustering with selected large-sized genes.

Results

1. Mucin phenotype is limited as a lineage marker because the markers change during progression.

The decrease in the G/GI-type and coincident increase in the I-type could reflect a loss of gastric phenotype from G and GI, respectively, secondary to an increase of in tumour size. There was no significant difference in the I-type frequency between the group A/B and group C tumours.

2. Quantitative PCR (qPCR) results

By comparing the PCR efficiencies between reference samples before and after WGA, we could demonstrate biased amplification; however, this bias was dependent on the examined genes and was cancelled by ratioing tumour and reference DNA copy numbers.

3. Genome-wide copy-number alteration patterns

The average T/R ratios of 30,098 gene regions were calculated from 55,023 probes. Based on the

（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。

(続 紙)

average T/R ratio, previously reported gains of 8q and 20 and loss of 5q were confirmed in our data and the frequencies of copy-number alterations (CNAs) did not significantly differ between groups A and B or between groups Cm and Cd, but they differed significantly between groups A or B and group C.

4. Repeated clustering with varying gene sizes revealed that the use of the genes that contain ≥ 4 probes was optimal. In this optimal condition, all the 43 tumours examined were classified into 3 major clusters: stable, unstable and intermediate.

The stable cluster comprised solely of group A/B tumours (4 and 7 in groups A and B, respectively) and accounted for 11 of the 19 (58%) group A/B tumours. In contrast, the unstable cluster alone included invasive carcinomas. In the stable cluster, no lesion exceeded 2 cm in diameter. Among the 3 group A/B tumour clusters, the tumour size distribution nearly overlapped, and there was no significant difference in the mean tumour size, suggesting that each cluster may represent an independent genetic lineage of different outcome.

5. The histological tumour grade or mucin phenotype of non-invasive neoplasms did not correlate with the clustering results.

6. Using a t-test, 51 genes with significantly different CNAs between the 3 clusters were identified.

Discussion

Mucin phenotyping demonstrated time-dependent losses of gastric-type lineage marker expression, and its utility may be limited with respect to outcome prediction. Therefore, we focused on aCGH approach.

Unsupervised clustering analyses of the array CGH data of 43 gland-forming gastric neoplasms disclosed 3 major clusters. The unstable cluster may represent a lineage of poor outcome, consisting of tumours from incipient to advanced stages, whereas the stable and the intermediate clusters, consisting only of intramucosal (group A/B) tumours, might not progress to invasive cancers. So-called adenoma-carcinoma sequence may occur within the unstable cluster, whereas it may be unlikely that the stable cluster accumulate CNAs to become unstable tumours because of opposing CNAs among the 3 lineages, and because, even in small non-invasive tumours, around two thirds of natural history of GC has already elapsed, and the tumours had already accumulated the CNAs sufficient for the determination of tumour outcome.

The determination of copy-numbers of the 51 genes, the cluster discriminators, could be used for the outcome prediction of endoscopically removed mucosal lesions, giving patients to chance for early detection of high-risk lesions and relieve patients with low-risk lesions from unnecessary surgical excision of stomach.

Conclusion

Outcome of individual tumours is not stochastically determined but can be predicted from the genomic copy-number profile even at the non-invasive stage. Since invasive carcinomas were included only in the unstable cluster, non-invasive neoplasms of the unstable cluster, accounting for 21% of non-invasive neoplasms, may accumulate genetic changes in a stochastic manner and progress to invasive GCs, whereas those of the stable cluster, accounting for 58% of non-invasive neoplasms may not. This classification was not significantly correlated with the histological grade, mucin phenotype or size of non-invasive tumours, but was consistent with the results of previous long-term follow-up studies.

別紙様式８（課程・論文博士共用）

学位論文審査の結果の要旨

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（学位論文審査の結果の要旨）（明朝体１１ポイント、６００字以内で作成のこと。）

胃の腺管形成性腫瘍性病変(非浸潤性腫瘍 19 例, 粘膜内癌 8 例, SM 以深浸潤癌 16 例)を対象として非浸潤性腫瘍が浸潤癌に進展するリスクを評価する研究を行った．腫瘍組織を形質発現から腸型，胃腸混合型，胃型と分類不能型に分類した．粘膜内腫瘍はウイーン分類にしたがって Group A(low grade)， Group B(high grade)と Group C(intramucosal invasive)に分類した．Microdissection system にて腫瘍細胞を採取し、抽出した DNA の全ゲノムを増幅後、array CGH 解析を行った．さらにクラスタ解析を行った結果から腫瘍を stable, unstable と intermediate に分類，ウイーン分類および形質発現分類と比較検討し，以下の点を明らかにした．

1) 粘膜内非浸潤性腫瘍 19 例の病理組織学的分類（ウイーン分類），形質発現分類とゲノムパターン分類の間に明らかな相関は認められなかった．
2) Group A は全て腸型であったが，それ以外では胃型形質を示すものが多くなった．
3) Group A の 7 例中 1 例，Group B の 12 例中 3 例が unstable なゲノムパターンを示した．

本論文は，胃粘膜内腫瘍の特性と進展の可能性について，新しい知見を与えたものであり，最終試験として論文内容に関連した試問を受け合格したので，博士（医学）の学位論文に値するものと認められた．

（総字数 598 字）

（平成 27 年 9 月 4 日）