Rapidly and slowly growing lineages in chromosomal instability-type gland-forming gastric carcinomas as revealed by multisampling analysis of DNA copy-number profile.

氏　　名	Duong Thanh Tu
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審査委員	主査　教授　扇田　久和
	副査　教授　谷　眞至
	副査　教授　九嶋　亮治

別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

※整理番号	850	（ふりがな） 氏　　名	ゆーんたーんとぅー Duong Thanh Tu
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Background and purpose: Gastric carcinoma (GC) is the fifth most common malignant neoplasm and the third leading cause of cancer-related deaths in both sexes worldwide. Recently, the Cancer Genome Atlas classification of GC includes four subtypes: Epstein-Barr virus (EBV)-positive GCs, microsatellite-unstable GCs, genomically stable GCs, and GCs with chromosomal instability (CIN). It remains unclear whether the most common CIN-type GCs are genetically homogeneous and how the risk of progression from early to advanced stage or metastasis risk is assessable. The extent to which early detection and treatment contribute to the reduction of mortality in these patients depends on the lineage continuity between the endoscopically resectable lesions and advanced cancer. In the present study, we utilized this approach to multiple samples from mucosal, extra-mucosal invasive and metastatic lymph node lesions of individual tumor specimens to confirm the consistency of individual-specific changes and to examine changes associated with tumor progression.

***Methods*:** This study used formalin-fixed paraffin embedded tissue specimens from 57 invasive gastric adenocarcinomas including 22 intramucosal, 6 submucosal and 29 advanced cancers. Of these tumors, 25 lymph node-positive (N+) and 22 node-negative (N0) tumors were surgically resected from 44 patients, and 10 mucosal GCs were removed by endoscopic dissection. For diagnosis of N0 tumors, 10 or more lymph nodes examined had to be free from metastasis. A total of 106 tumor samples were taken from mucosal, extra-mucosal invasive, and lymph node lesions by laser micro dissection. The DNAs extracted from tumor and normal tissues were used for array CGH. Then, the data were analyzed by clustering analyses and penetrance plots. EBV status was determined by EBV-encoded small RNA (EBER) *in situ* hybridization. The two monoclonal antibodies against two mismatch repair (MMR) proteins were immunohistochemically used to assess enzyme silencing, which is closely related to microsatellite instability (MSI): MSH6 and PMS2. Mucin phenotype was analyzed immunohistochemically using monoclonal antibodies against MUC2, MUC5AC,

（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を用いて印字すること。

２．※印の欄には記入しないこと。

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（続紙）

CD10. A monoclonal antibody to p53 protein was used to assess p53 expression pattern.

Results:

- A total of 106 samples were classified into two major clusters: A and B. Clusters A and B were characterized by the presence of gain-rich and loss-rich areas, respectively. Between these two clusters, there were significantly different in tumor locus and patient age. The T1/T2–4 ratio, the frequency of small cancers (diameter ≤2–4 cm), and intestinal mucin expression were higher in cluster B than in cluster A.
- There were no differences in the frequencies of MMR silencing, mutant p53 pattern, and lymph node metastasis between the two clusters. Losses of mismatch repair protein samples commonly showed dark in the heat map but were not distributed in a cluster. The tumors we examined in this study contained only 1 case with EBV infection.
- The penetrance plots of the tumors with retention of MMR enzyme expression showed conspicuous gains and losses which were divided into two patterns by clustering. The changes common to both clusters were 8q+, 13q+, 20q+, and 5q−. Clusters A and B showed invasion/metastasis-related chromosomal gains (7p/q+, Xp/q+, etc.) and losses (4p/q−, etc.), respectively. The MMR-silenced tumors showed a small number of chromosomal gains, including 12p/q+.
- We identified 32 genes that significantly contributed to the difference in clusters A and B.

***Discussions and Conclusions*:**

- The age difference might be explained by the difference in the frequency of early GCs, whereas the locus difference suggests that this clustering might reflect distinct genetic lineages. This was also supported by mucin phenotyping; progression-independent intestinal expression was significantly different between clusters A and B.
- Comparison of other clinicopathological factors between clusters A and B indicated that the tumors in cluster A was more rapidly growing (larger, deeper tumors) than those in cluster B. ESD specimens were mostly included in the latter. These findings confirm the safety of ESD treatment but imply that early detection and endoscopic treatment are more difficult in cluster A than in cluster B tumors. Using 32 selected genes that showed significantly different copy numbers between clusters A and B, a tumor may be classified into 2 clusters. This information can be taken into consideration in treatment of early GCs.
- There was no significant differences in the lymph node metastasis risk between the rapidly and slowly growing lineages, whereas there were lineage-specific, invasion/metastasis-related chromosomal changes. These findings suggest that metastasis risk is determined not at an earlier but at a later stage during tumor development.

別紙様式８（課程博士・論文博士共用）

学位論文審査の結果の要旨

整理番号	850	氏　　名	Duong Thanh Tu
論文審査委員			

（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

胃癌は４つのサブタイプに分類されるが、染色体不安定型胃癌が遺伝的に均一なものか、また、このサブタイプで早期癌から進行癌や転移巣形成へと進展するリスクはどのようなものかは不明である。本論文では、浸潤性の胃腺癌 57 症例 106 組織標本を用いて検討を行い、以下の点を明らかにした。

1) 胃癌組織標本を DNA コピー数プロファイル解析で、コピー数増加の多いクラスターA と減少の多いクラスターB に分類した。
2) この両クラスター間で、胃癌存在部位、患者年齢に有意差があり、また、T1/T2-4 比、小サイズ癌（直径 2-4 cm 以下）頻度、腸型ムチン発現に関し、クラスターB の方がクラスターA よりも上昇していた。
3) ミスマッチ修復（MMR）、p53 変異パターン、リンパ節転移の頻度について両クラスター間で差はなかった。
4) クラスターA では、がんの浸潤・転移に関連する染色体変化（7p/q+、Xp/q+など）が見られ、クラスターB では、4p/q-などの染色体変化が見られた。
5) クラスターA と B の違いを決定づける 32 遺伝子を同定した。
6) クラスターA の方がクラスターB よりもがんの進展は早いことが示唆されたが、がんの転移リスクに差はなかった。

本論文は胃癌の進展について新たな知見を得たものであり、また、最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

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