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学 位 論 文 題 目	Application of a Novel Method of Double APAAP Staining With Subsequent Quantitative Image Analysis to the Examination of Integrin Expression in Undifferentiated-type Gastric Carcinomas (アルカリホスファターゼ抗アルカリホスファターゼ二重染色法と画像計 量の未分化型胃癌でのインテグリン発現解析への応用。)
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学位論文題目	Application of a Novel Method of Double APAAP Staining With Subsequent Quantitative Image Analysis to the Examination of Integrin Expression in Undifferentiated-type Gastric Carcinomas		
<p>【研究の目的】 To disclose the pattern of integrin phenotype (IP) alterations of during the progression of undifferentiated-type gastric carcinomas (UGC).</p> <p>【方法】 We used double alkaline-phosphatase anti-alkaline-phosphatase (APAAP) staining as the most sensitive: 1) the 1st step – staining with one of 11 mouse anti-integrin antibody ($\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, αV, $\beta 1$, $\beta 4$, $\alpha V\beta 3$, $\alpha V\beta 5$, $\alpha V\beta$), 2) the 2nd – staining with mouse anti-poly-cytokeratin antibody. Microwave boiling was performed between the first and the second steps to avoid the antibodies cross-reactivity. The purpose of the second step was to include in calculations only cancer cells, which in UGC could be mixed with inflammatory cells in early carcinomas and stromal cells in deeper parts. Microphotos were taken after each step and merged by computer to obtain “immunofluorescent-like” images. 4-5 fields in different tumor depth of 27 UGC and lymph node (LN) metastasis were examined to calculate the fraction of integrin-positive cells among 100 of cytokeratin-positive cancer cells.</p> <p>【結果】 1. In non-neoplastic (NN) stomach mucosa some epithelial and ubiquitous ($\alpha 2$, $\alpha 6$, and $\beta 1$) integrin subunits (IS) showed a uniformly positive pattern, whereas others ($\alpha 3$ and $\beta 4$) showed vertical gradients of distribution. Mesenchymal IS ($\alpha 1$, $\alpha 5$) were not expressed. αV-family integrins were observed in areas of reactive changes.</p>			

(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. During deeper invasion of UGC the expression of:
 - a) epithelial and ubiquitous IS showed significant trends toward a decrease,
 - b) mesenchymal and α V-family (except α V β 6) integrin and IS was enhanced significantly.
3. The expression of α V β 6 integrin was significantly upregulated in LN metastasis.
4. The expression of mesenchymal IS was significantly higher in the cells invading through the muscle than in those invading through the perimysium.

【考察】

Our present results concerning integrin expression in NN stomach epithelia have reconfirmed and expanded most of the previous data that were based on IF- and horse-radish-peroxydase-based studies.

We have demonstrated the trend that the deeper UGC invaded in the stomach wall, the less epithelial IS and more mesenchymal IS of β 1 family were expressed. This trend may reflect EMT, which is common in tumor progression. Integrin EMT was most prominent in cancer cells migrating through the smooth-muscle cells of the muscularis propria, suggesting the necessity of the fibroblast-like motility mechanism for such movement.

The trend of gradual enhancement of α V family integrin expression as the tumor invades deeper may reflect the notion that integrins of this family are linked not only with carcinogenesis but also with UGC tumor progression and histological pattern formation.

【結論】

Our results have demonstrated that the novel method of double APAAP staining with subse-quent quantitative analysis of digital images is applicable to analyses of integrins expression in UGC, in which the recognition of cancer cells is not easy.

In comparison with routine IHC/IF staining, this method has allowed us to overcome the problem of crossreactivity in double staining, to produce more-quantitative results, thereby we could demonstrate EMT and integrin other repertoire alterations in tumor progression.

学位論文審査の結果の要旨

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論文審査委員			
(学位論文審査の結果の要旨)			
<p>未分化型胃癌は、間質と接する面が多く、癌細胞と間質との相互作用の研究に適しているが、腫瘍細胞と間質細胞との識別が容易でなく、上皮間葉転換の研究には工夫が必要であった。本研究では、上皮間質間相互作用で重要な働きをしているインテグリンに着目し、同一切片で sequential にインテグリンと上皮マーカーの二重免疫染色を行い、各ステップの画像をコンピュータ上で重ね合わせ、定量的解析を行なう手法を開発した。これを個々の腫瘍の複数個所に用いて、癌進展に伴い、上皮インテグリンの発現低下と、間葉インテグリン、がん胎児性インテグリンの発現亢進を明らかにした。これは、未分化型胃癌の進展に上皮間葉転換が重要な役割を果たしていることを示唆している。</p> <p>本論文は、上皮間葉間相互作用の研究に有効な方法論を開発し、それをを用いて腫瘍進展の研究に新たな知見を加えたものであり、最終試験として論文内容に関連した試問を受け、博士(医学)の学位論文に値するものと認められた。</p>			
(平成 22 年 1 月 26 日)			