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Integrin and E-Cadherin Expression Alterations as a Possible Reason of Undifferentiated-Type Gastric Carcinoma Diversity

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1. Introduction

Undifferentiated-type gastric carcinoma (UGC), according to the Japanese classification of gastric carcinoma (GC) (Japanese Gastric Cancer Association, 1998), is a type that poorly develops a tubular component. Relationship between Japanese and other classifications was discussed previously (Natsagdorj et al., 2008). The etiology and histogenetic pathways of UGC are still less elucidated than in GC with predominant tubular component (differentiated type GC, DGC), despite the notable advances of molecular technology and remarkable increase of percentage of UGC in the whole GC pool due to decrease of DGC and growth of UGC incidence worldwide in the last two decades (Crew & Neugut 2006; Kaneko & Yoshimura, 2001; Sidoni, 2005).

Histological structure of UGCs is also more complicated than that of DGC and trends to display remarkable diversity due to combination of signet-ring cells, poorly differentiated, tubular and mucinous components; cohesive and dissociative cell arrangement; areas with scirrhous and non-scirrhous stroma. Additionally, cancer cells are intermingled with inflammatory cells (especially in mucosal area of early UGC) and stromal cells (especially in extramucosal areas).

Possible reason of UGCs histological diversity is contribution of at least two processes to UGC local growth and invasion, i.e. individual migration of dissociative cells and cohort-type migration of cell-clusters (Friedl & Wolf, 2003; Nabeshima et al., 1999). It could be supposed, that both individual and cohort migrations depend on cell-extracellular matrix (ECM) interaction and could be studied from the position of cell-ECM receptors integrins. Cohort-type migration demands, additionally, "localized modulation of cell-cell adhesion" (Friedl & Wolf, 2003; Nabeshima et al., 1999), which could be changed allowing cell groups or scanty tubular structures to penetrate environmental tissues. Such cell-cell adhesion could be studied from the position of cadherin-phenotype alteration.

Integrin and E-cadherin phenotype was proved to undergo remarkable changes during tumor progression of GC and other carcinomas, being related to various clinicopathological tumor features linked with tumors invasive properties (Choi et al., 2009; Hazan et al., 2004; Stefansson et al., 2004; Yanchenko et al., 2009; Yang et al., 2008). However, most studies were performed at the tumors array without specification of their individual features and the precise interrelationship between integrin and cadherin phenotype alteration and UGCs histopathological diversity has not been elucidated yet.

Why is the precise analysis of UGCs histology so important? Tumor invasion and growth is a process, however in pathology we commonly deal with its result, i.e. tumor with its individual features. Some of those features, e.g. scirrhous stroma, were already proved to be linked with high invasiveness and poor prognosis (Guszczyń & Sobolewski, 2004).

However, the role and prognostic significance of other tumor components, for example signet-ring cell (SRC) component in tumor progression is still unclear. Whereas early SRC carcinomas are rather dormant tumors with predominantly spreading growth, advanced SRC are linked with LN prominent LN metastazing and poor outcome (Humar et al., 2007; Hyung et al., 2002; Kim et al., 1997; Li et al. 2007).

One more example is the area of layer arrangement of SRC and poorly differentiated cells, called layered structures (LS), in mucosal areas of some UGCs. LSs have been proved to be the only one reliable histological sign of primary genesis of UGC from early SRC carcinomas. UGCs aroused from dedifferentiated tubular GC lack LS (Humar et al., 2007; Natsagdorj et al., 2008; Sugihara et al., 1987). Biological behavior of advanced GC arisen from early SRC (primary UGC) and via tubular GC dedifferentiation was proved to be different (Natsagdorj et al., 2008), and it could be supposed that presence and extension of LS could predict tumor aggressiveness.

To clarify the invasive potential of UGC and interrelationship between tumor histology and cell-cell and cell-ECM interaction alterations we introduce precise quantitated analysis of histological structure of each individual UGC case, supplemented by integrated immunohistochemical analysis of integrin and E-cadherin phenotype alterations.

2. Materials and methods

2.1 Materials

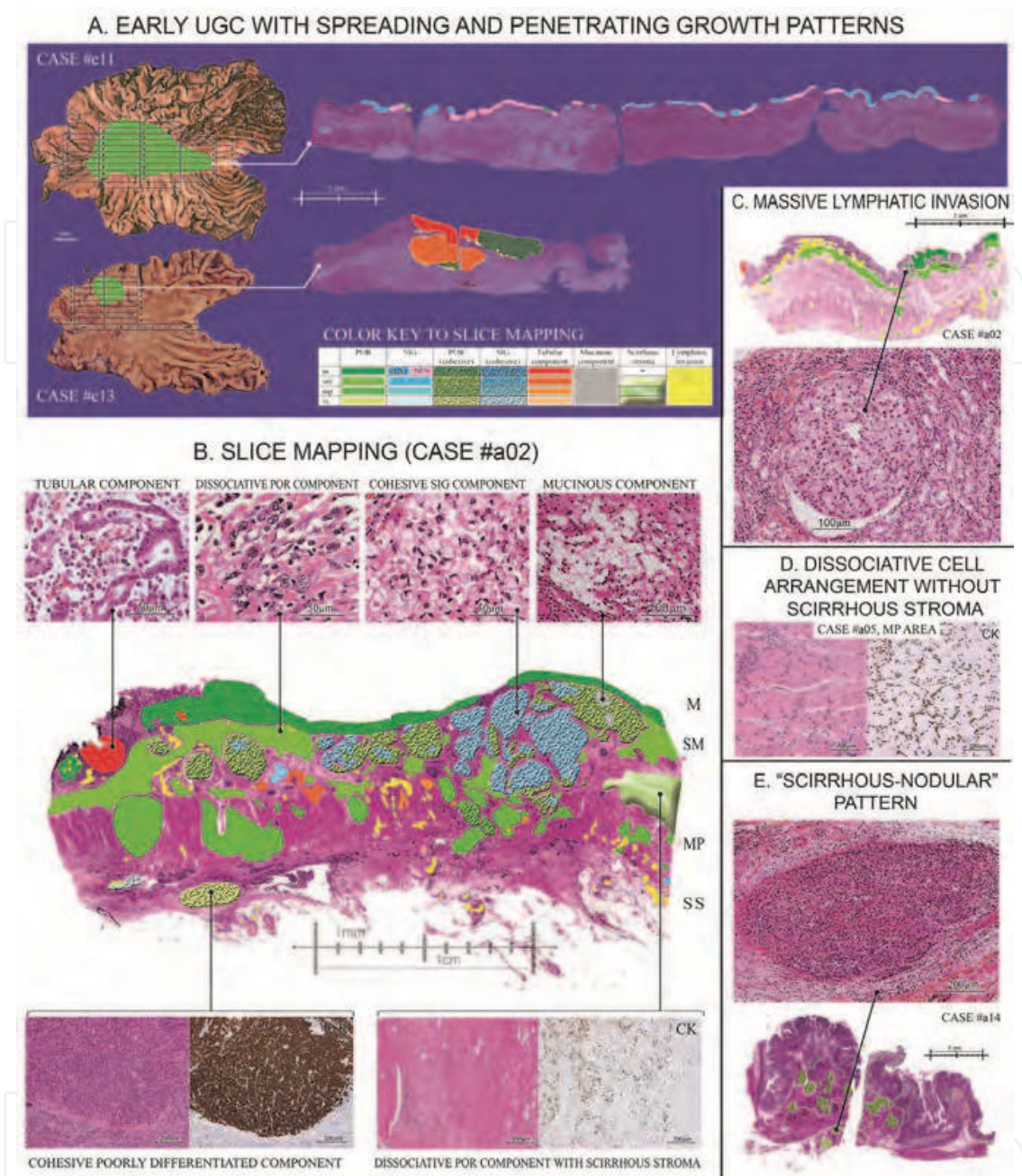
We used 30 randomly selected cases with fresh resection specimens of UGC (13 early and 17 advanced). In early UGC group 10 cases were diagnosed as signet-ring cell carcinomas (SIG) and demonstrated LS in the mucosa; 3 cases were poorly differentiated adenocarcinoma (POR), one of them with remarkable tubular component. In advanced UGC group 1 case was diagnosed as SIG and 16 as POR, most of which were accompanied with some SRCs and/or tubular component. Nine of advanced UGC had remnants of LS in the mucosa.

To study integrin expression, tissue samples were snap-frozen in liquid nitrogen and then stored at -80°C until sectioning. Serial 4- μm -thick sections were cut at -22°C and placed in plastic boxes for storage at -80°C until further processing. To study expression of other antigens, we used 10% formalin-fixed paraffin-embedded tissues and cut them into 2- μm -thick sections.

2.2 Method

2.2.1 Quantitative tumor structure analysis

To analyze tumor histotype, each tumor was cut through into 0.5×2-3 cm blocks (Fig. 1A), the entire HE (hematoxylin and eosin) stained 2 μm -thick slices (one from each block) was scanned using Nikon Super COOLSCAN 5000 ED film scanner at a resolution of 2000 dpi. Scanned slice area was mapped at the microscope control (Fig. 1A, B, C) according to: (1) invasion depth (mucosal, submucosal [sm], muscularis propria [mp] and subserosal /adventitial [ss] areas); (2) tumor histotype (signet-ring cell, poorly differentiated, tubular and mucinous components); (3) cell cohesiveness (cohesive and dissociative component); (4) stromal development (scirrhous and non-scirrhous areas). Lymphatic and venous invasion areas were marked separately. Different tumor areas were assessed using analysis/measuring feature of Adobe Photoshop CS4 Extended Edition (Adobe Systems,



A - Early UGC with spreading (#e11) and penetrating pattern of invasion. Green areas at the photos of stomachs at the left designate tumor invasion areas. Mapped slices at the right demonstrate superficial (mucosal) spreading of relatively dormant case #e11, vs. rapid sm penetration of case #e13. Color key to slices mapping refer to all pictures (A, B, C, E). **B** - example of slices mapping demonstrate the histological structures designated as tubular, poorly differentiated (POR), cohesive signet-ring cell (SIG) and other components. Cytokeratin (CK) staining (as well as in picture D) supplemented HE slices when identity of tumor cells is not obvious. M, SM, MP, SS - invasion depth. **C** - unusually extensive lymphatic penetration (mapped in yellow). Cells in the lymphatic vessels are SRC (lower microphoto), although tumor consists mostly of POR component (mapped in green). **D** - case demonstrating that dissociative cell arrangement is not always means scirrhous stroma development. **E** - case demonstrating rather rare mixture of nodules with cohesive cell arrangement and areas with scirrhous stroma.

Fig. 1. Blocks and slices mapping; examples of UGCs structure.

Mountain View, CA). “Vertical” invasion area (Area V) was calculated as a sum of all slices areas. The percentage of each area was calculated as a percentage of the corresponded area in the whole tumor area at this slice, e.g.

$$\text{Percentage of mucosal area} = \frac{\text{mucosal area}}{\sum \text{mucosal, sm, mp and ss areas}}$$

We also assessed some other clinicopathological UGC features related to tumor invasiveness, such as: (1) “horizontal” spreading area (Area H); the spreading area at each slice was mapped at stomach wall photograph and then assessed using analysis/measuring feature of Adobe Photoshop (Fig. 1A); (2) the number of lymph node (LN) metastasis, (3) invasion front shape (α , β , γ -invasion according to Japanese classification of GC) (Japanese Gastric Cancer Association, 1998).

2.2.2 Immunohistochemical study of integrin expression (frozen section processing)

To study integrin expression we used double APAAP staining with subsequent image analysis, which was introduced earlier (Yanchenko et al., 2009). Briefly, acetone fixed frozen sections were stained for integrin and, after heating, for cytokeratin (CK) to discriminate cancer cell from inflammatory and stromal cells. Microphotos were taken after first and second steps and processed with Adobe Photoshop. For the estimation of the fraction of positive cells, cancer cells positive for integrin were counted within 100 cytokeratin-positive cancer cells, in mucosal, submucosal and deeper areas.

2.2.3 Immunohistochemical study of integrin expression (paraffin-embedded section processing)

Automated staining using Ventana Discovery XT autostainer (Ventana Japan, Yokohama, Japan) was performed for staining of E-cadherins. As HECD-1 antibody, often used for E-cadherin extracellular domain staining, could not be used for automated staining, it was replaced by G-10 (Table 1), the results were comparative with HECD-1 manual staining with autoclave-based antigen retrieval in citric acid.

To discriminate cancerous cells from non-cancerous cells in UGC, where cancerous cells are often intermingled with stromal and inflammatory cells, we used an epithelial marker cytokeratin, as was described previously for integrin study (Yanchenko et al., 2009). After automated cadherin staining slices underwent 15 minute trypsin-based antigen retrieval (trypsin was obtained from Histofine, Tokyo, Japan) with subsequent overnight anti-cytokeratin antibody incubation.

Since E-cadherin was expressed only by epithelial cells, we could omit image processing, necessary for study of mesenchymal and ubiquitous proteins (Yanchenko et al., 2009), and it was possible to estimate the fraction of cadherin-positive cells within 100 cytokeratin-positive cancer cells in mucosal, submucosal and deeper areas without taking intermediate microphotograph.

Antibodies, used for integrins and E-cadherin staining are listed in Table 1.

Controls. For a positive control we used: (1) normal tissue of the same stomach sample (for proteins which normally occur in stomach tissue), (2) specimen from our collection, proved to be positive for this protein (for abnormal proteins). For a negative control, every

processed glass contained one serial section of the same sample (frozen tissues) or we used separate slice of the same sample (paraffin-embedded tissues), which was stained with an omission of the primary antibody.

Antigen	Clone	Dilution	Manufacturer
α 1 IS	TS2/7	1:50	Santa Cruz Biotechnology, Inc, Santa Cruz, CA
α 2 IS	P1E6	1:200	Chemicon International, Inc., Temecula, CA
α 3 IS	J143	1:40	GeneTex, Inc., San Antonio, TX
α 5 IS	SAM-1	1:10	Chemicon International, Inc., Temecula, CA
α 6 IS	4F10	1:20	Chemicon International, Inc., Temecula, CA
α V IS	LM142 13C2	1:1000 1:20	Chemicon International, Inc., Temecula, CA
β 1 IS	K-20 DF5	1:50 1:100	Santa Cruz Biotechnology, Inc, Santa Cruz, CA BIOMOL International, L.P., Plymouth Meeting, PA
β 4 IS	3E1	1:2500	Chemicon International, Inc., Temecula, CA
α V β 3 integrin	P1F6	1:1000	Chemicon International, Inc., Temecula, CA
α V β 5 integrin	LM609	1:200	Chemicon International, Inc., Temecula, CA
α V β 6 integrin	E7P6	1:200	Chemicon International, Inc., Temecula, CA
E-cadherin ^{ID}	AEC-36	1:1500	BD Biosciences, San Jose, CA
E-cadherin ^{ED}	HECD-1	1:500 1:50	Takara Shuzo CO., LND, Otsu, Japan Abcam, Cambridge, MA
E-cadherin ^{ED}	G-10	1:100	Santa Cruz Biotechnology, Inc, Santa Cruz, CA
Cytokeratins (1-8,10, 14-16, 19)	AE1/AE3	prediluted	Histofine, Tokyo, Japan

Table 1. Primary mouse antibodies. IS - integrin subunit, ID - intracellular domain; ED - extracellular domain.

2.3 Calculation and statistical analysis

“Normal” or “preserved” expression means that more than 95% of tumor cells expressed this receptor at the same manner as NN epithelium (positive for normal proteins and negative for abnormal). “Weak alteration” means that no more than 1/3 (0-33%) of tumor cells demonstrated altered expression pattern. “Moderate alteration” designates alteration in 34-66% of tumor cells, and in cases with “strong alteration” the percentage of abnormally expressing cells is more than 2/3 (>67%).

Descriptive statistical parameters, including means and standard errors, were calculated with the standard method (using descriptive statistics package, Microsoft Excel). To compare early and advanced UGCs, the statistical significance of differences between means

was calculated using Student's unpaired t-test. To prove the mutual correlation between protein expression and UGC features, correlation coefficient R was calculated (using the same statistics package, Microsoft Excel). Mean values throughout this report are expressed as means \pm standard error. A probability (P-value) less than 0.05 was considered significant in all methods, including correlation analysis.

3. Results

3.1 Histological structure of UGC: UGC diversity analysis

3.1.1 Invasion depth

According to the invasion depth pattern, UGC could be divided into several groups (Fig. 2): Early UGC: 1) E(m) - intramucosal tumors, submucosal (sm) involvement less than 1% of the total tumor Area V (12 cases); 2) E(sm) - predominantly intramucosal with some submucosal invasion (1 case).

Advanced UGC: 3) A(sm) - mostly submucosal (3 cases), 4) A(sm/mp) - with equal submucosal and muscularis propria (mp) portions (9 cases), 5) A(mp) - with prominent muscularis propria portion (5 cases).

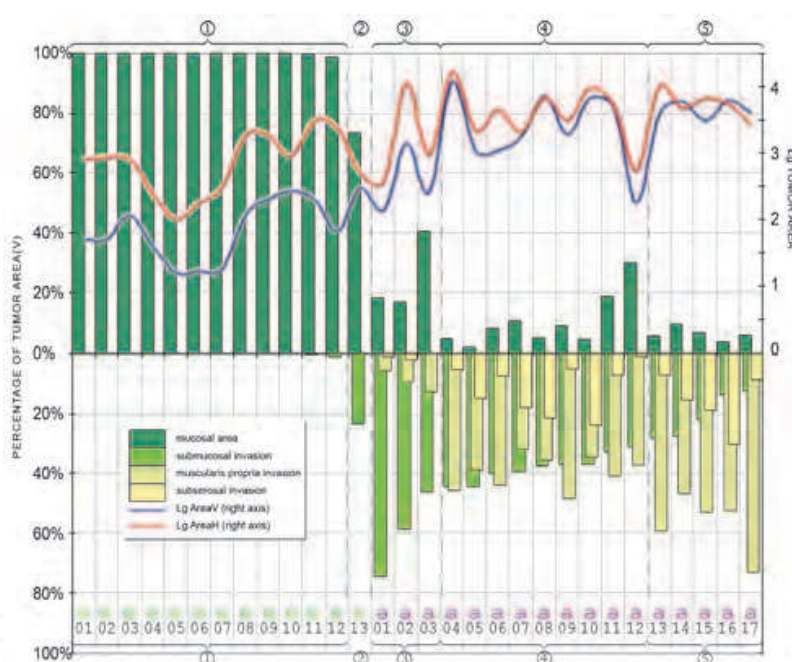


Fig. 2. Invasion depth patterns All UGC cases are arranged along x-axis. Cases are divided into 5 groups according to the proportion of m, sm, and mp component in their structure. Bars depict the percentage of correspondent components in whole tumor. Bars are arranged above and below x-axis only for better representation, i.e. location below the axis does not mean negative values. Line graphs depict tumor size and are plotted at secondary (right) y-axis.

3.1.2 UGCs histotype

3.1.2.1 Signet-ring cell and poorly differentiated components, layer structures

Histotype of tumor was as follows (Fig. 3): (1) SIG - predominantly signet-ring cell (SRC) tumors, were SRC comprised more than 55% of carcinoma area: 11 cases, among them 10

early and 1 advanced cases (77% of early and 6% of advanced UGC, respectively); (2) POR – predominantly poorly differentiated tumors (poorly differentiated cells comprised more than 55% of carcinoma area): 19 cases, 3 early and 16 advanced (23% of early and 94% of advanced UGC, respectively).



Fig. 3. UGCs histotypes (for explanation see Fig. 2). Enclosed crosses depict percentage of LS in mucosal part.

Ten (77%) early UGCs and 9 (53%) advanced UGCs demonstrated layer structures (LS) in the mucosa (Fig. 3). However, in early carcinomas LS formed the most part (88%±5%) of tumor area, whereas in advanced carcinomas they were replaced by poorly differentiated component and remained only in 5% ± 3% of mucosal part.

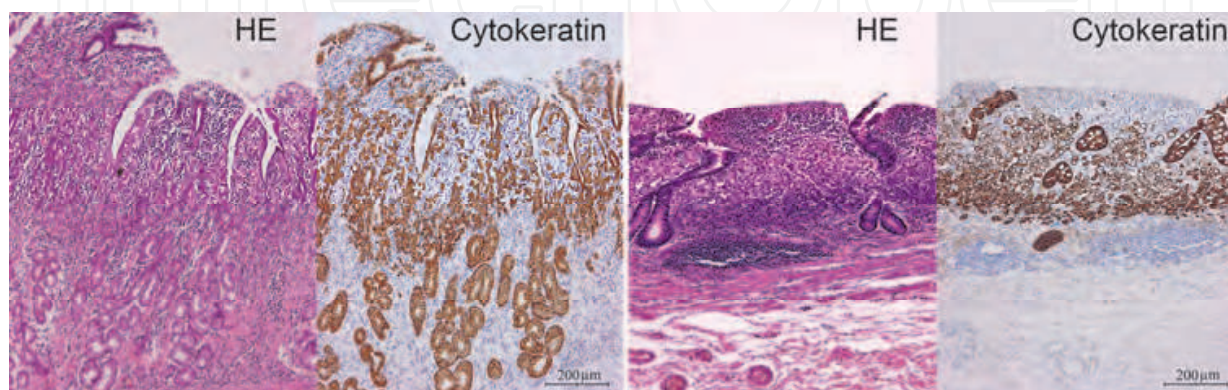


Fig. 4. Variants of layered structures in early carcinomas.

A SRC component percentage correlated in early UGC with bigger tumor horizontal invasion area ($R=0.58$) and spreading growth ($R=0.71$) (see Fig. 1A, case #e11) submucosal invasion risk in such tumors was lower ($R=0.55$). On the other hand, in advanced UGC SRC was linked to smaller tumor size ($R=0.60$), and spreading in the submucosa ($R=0.71$), but not to the muscularis propria ($R=0.64$) (compare Fig. 2 and Fig. 3, cases #a01, #a03).

In advanced UGC LS remnants percentage in mucosa correlated with smaller tumor size (Area V, $R=0.54$) and mucosal vs extramucosal portion prevalence ($R=0.76$), those tumors had smaller tendency to invade muscularis propria ($R=0.49$) (compare cases #a03, #a05, #a06 with LS remnants and cases #a14-16 without LS at Fig. 2 and Fig. 3)

Only in advanced UGC without LS in the mucosa, a SRC component correlated with lymphatic invasion ($R=0.99$), venous invasion ($R=0.82$) and LN metastasis number ($R=0.71$). In one of such tumors lymphatic invasion comprised 13% of the whole tumor area and consisted mostly of SRC, whereas the whole tumor was mostly poorly differentiated (Fig. 1C, Fig. 3 case #02).

3.1.2.2 Tubular component

A tubular component (TC) was found in 5 (38%) early and 10 (59%) advanced UGC and ranged from 0.01% to 41.46% of the whole tumor area (Area V) (Fig. 3). In early UGC TC was higher in bigger tumors ($R=0.55$), with tendency to submucosal and lymphatic vessel invasion ($R=1.00$), and less layer structures portion ($R=0.55$). In advanced UGC presence of TC did not correlate with local invasion features, but was linked to bigger number of LN metastasis ($R=0.54$).

3.1.2.3 Mucinous component

A mucinous component was found in 2 (15%) early and 7 (41%) advanced UGC ranged from 0.03% to 6.62% of the whole tumor area (Area V) (Fig. 3). In advanced UGC it appeared mostly in tumors with developed SRC component ($R=0.57$).

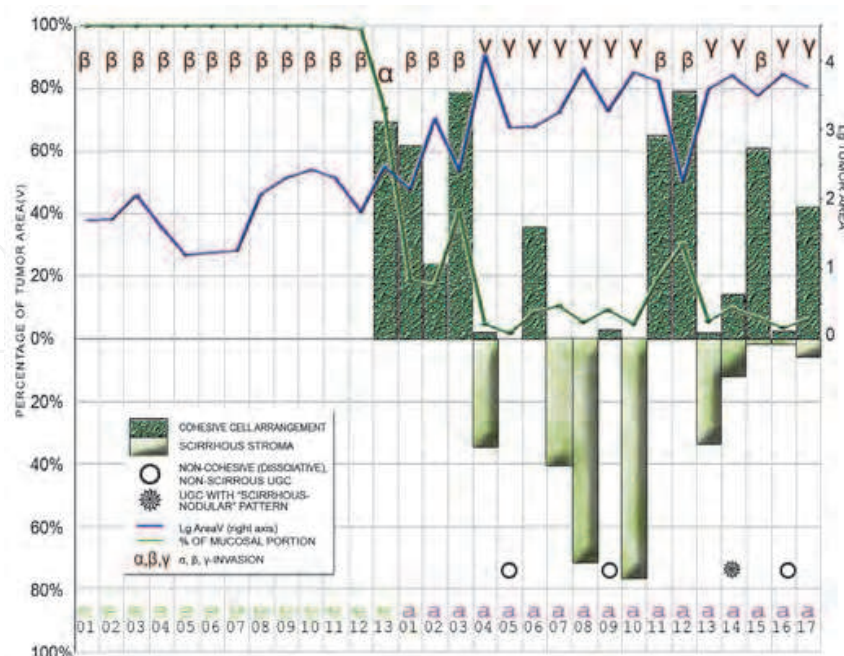


Fig. 5. Cohesive cell arrangement, scirrhus stroma, and invasion front (for explanation see Fig. 2)

3.1.3 Cell cohesiveness

Areas with cohesive cell arrangement were observed in 2 (15%) of early and 15 (88%) of advanced UGC, ranging from 1% to 79% of the whole tumor area. 1 (8%) early and 5 (29%) advanced UGC were predominantly (>60% of Area V) cohesive (Fig. 5). As could be expected, advanced UGC with bigger cohesive component were smaller in size (Area V; $R=0.64$), had higher mucosal (vs extramucosal) portion ($R=0.77$) (at Fig. 5 compare sizes and mucosal portion of cases a#03 and a#12 with “pure dissociative” cases #a08 and #a10). The only early case #e13 with cohesive poorly differentiated growth had the highest percentage of tubular component (Fig. 3), and demonstrated the biggest submucosal invasion portion (Fig. 2), despite rather small horizontally spreading area (Fig. 1A).

3.1.4 Stromal component

Scirrhou component was present in 10 (59%) advanced UGC and was prominent (>30% of the whole tumor area) in 5 of them, correlating with bigger tumor size (Area V; $R=0.50$) (Fig. 5, compare cases #a04, #a07, #a08 and #a10 with other advanced UGC).

Despite scirrhou component inversely correlated with cohesive growth pattern ($R=0.55$), and it could be seen from the Fig. 5 that in most tumor of cohesive cell arrangement and scirrhou stroma were mutually exclusive components, non-cohesive cell arrangement did not always mean scirrhou stroma development, and 3 of advanced UGC were mostly (>98%) non-cohesive, but did not demonstrate scirrhou stroma (Fig. 1D, Fig. 5, cases a#05, a#09, a#16). Additionally, case #a14 partially demonstrated a pattern that could be named “scirrhou-nodular”, where nodules of cohesive tumor cells were surrounded by scirrhou stroma. (Fig. 1E, Fig. 5).

3.1.5 Invasion front

Invasion front was clearly seen in all slices (α -INF) only case #e13, mostly cohesive tumor, other early UGC demonstrated fuzzy infiltration front (β -INF) (Fig. 5). 6 (35%) of advanced UGC, mostly with prevailed cohesive component, had clearly seen invasive front in cohesive areas and fuzzy or indistinguishable front in non-cohesive areas (β -INF). Other advanced UGC exhibited γ -INF and most of their borders were unclear (Fig. 5).

Less distinct borders / invasion fronts correlated not only with dissociative growth pattern (in advanced UGC), but with such signs of local invasion tendency as bigger tumor size ($R=0.60$); and extramucosal (vs mucosal) growth prevalence ($R=0.66$) (Fig. 5).

3.2 E-cadherin expression and UGC histopathological features

Average percentage of E-cadherin positive cells (Table 2) had no significant difference in early and advanced UGC.

	E-cadherin (internal domain)	E-cadherin (external domain)
NN expression pattern	strongly positive	moderately positive, except some surface cells
Early UGC	0.84±0.08	0.44±0.08
Advanced UGC	0.56±0.11	0.28±0.07
T-test	NS	NS

Table 2. Percentage of cancer cells positive for internal and external domains of E-cadherin

However, the pool of UGC was rather heterogenous in E-cadherin preservation or loss (Fig. 6). Around a half of UGC (early and advanced) preserved normal expression of internal domain, and around a quarter of them preserved normal pattern of external domain expression (Fig. 6, see first bars at graphs). On the other hand, almost quarter of cases (23%: 1 (8%) early, 6 (35%) advanced) showed strong loss of internal E-cadherin domain expression and almost half of cases (47%: 4 (31%) early, 10 (59%) advanced) demonstrated strong loss of external domain (Fig. 6, see last bars at graphs).

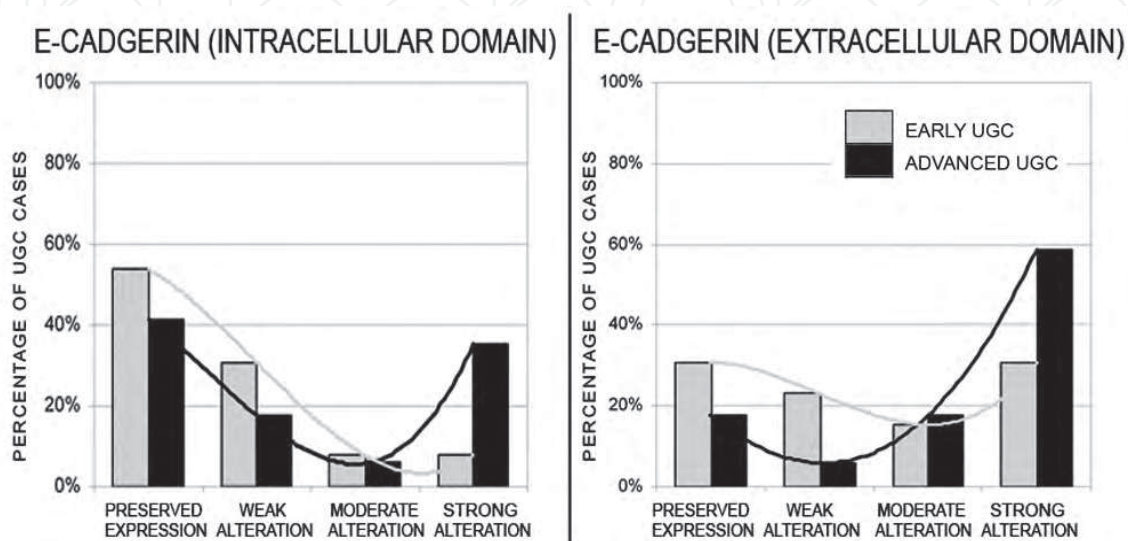


Fig. 6. UGC distribution according E-cadherin expression pattern. All UGC cases are arranged along x-axis. Bars represent percentage of UGC cases in the whole UGC group (e.g. 7 of 13 early UGC demonstrate normal expression of external E-cadherin domain, so the correspondent bar equals 54%). Lines are trends of cadherin and integrin distribution.

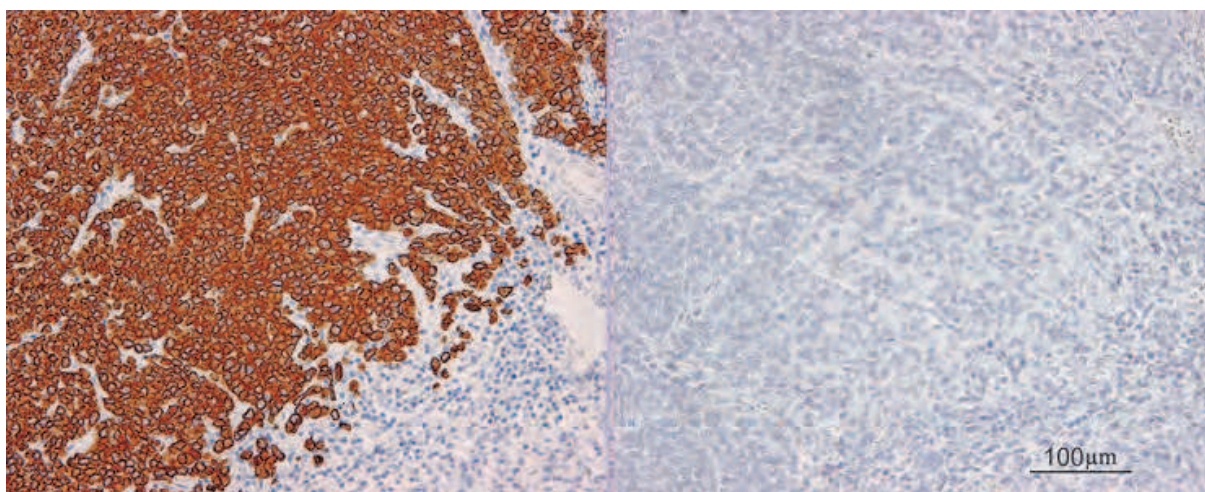


Fig. 7. Loss of E-cadherin expression in cohesive tumor. Left: cyokeratin staining. Right: same area, stained for E-cadherin (internal domain).

Tumors with preserved E-cadherin expression (both domains) trended to have smaller size (AreaV, $R=0.37$), with less mp portion ($R=0.36$). External domain of E-cadherin was stronger preserved in those advanced UGC with LS, which demonstrated minor tubular component

(R=0.73) (such as the case a#08, Fig. 3). Intriguingly, the tendency to lose internal domain was stronger in all tumors with more prominent cohesive component (R=0.41), all advanced UGC with total E-cadherin loss were mostly cohesive [Fig. 7 and Fig. 3, cases a#03, a#06, a#11, a#12, a#15].

Loss of external and internal E-cadherin domains correlated with each other (R=0.78), being more severe for external domain (Fig. 6).

3.3 Integrins expression and UGC histopathological features

Non-neoplastic (NN) pattern of integrin expression was described earlier (Yanchenko et al., 2009), briefly see Table 3.

	α6	a2	b1	b4	α3	a1	a5	aV	aVb5	aVb3	aVb6
NN	+++	+++	+++	G+	G+	-	-	+/-	+/-	-	-
Early UGC	0.85± 0.04	0.75± 0.03	0.87± 0.05	0.73± 0.05	0.83± 0.03	0.04± 0.01	0.12± 0.03	0.30± 0.06	0.11± 0.03	0.04± 0.02	0.10± 0.04
Advanced UGC	0.87± 0.02	0.72± 0.05	0.84± 0.03	0.65± 0.05	0.71± 0.06	0.19± 0.03	0.13± 0.02	0.47± 0.08	0.28± 0.06	0.07± 0.03	0.10± 0.03
T-test	NS	NS	NS	NS	NS	<0.01	NS	NS	<0.05	NS	NS

"+++” - expressed strongly in all epithelial cells, “G+” - expressed mostly in glandular portion, “-” - no expression, “+/-” - expressed in areas of plastic changes.

Table 3. Percentage of integrin positive cells

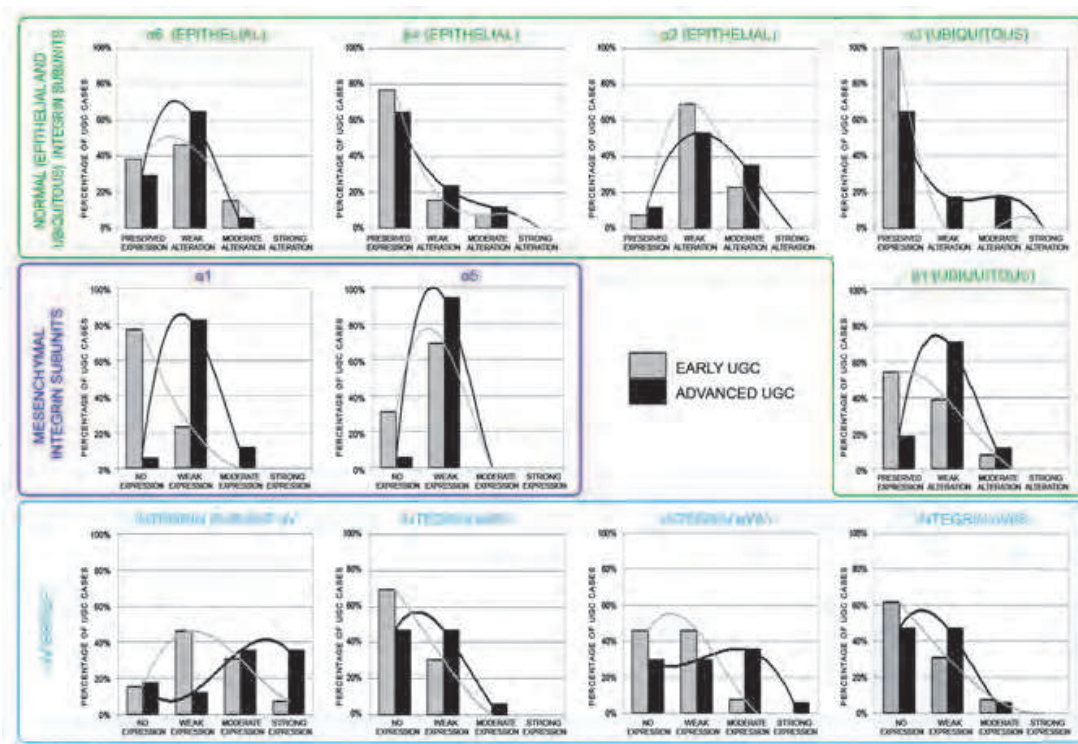


Fig. 8. UGC distribution according integrin expression pattern.

3.3.1 Epithelial integrins

All integrin subunits, common for normal stomach epithelium ($\alpha 2$, $\alpha 3$, $\alpha 6$, $\beta 1$, $\beta 4$) similar to internal domain of E-cadherin, demonstrated strong trend to be preserved both in early and advanced UGC (Table 3). However, unlike E-cadherin, none of the UGC cases demonstrated total loss of any normal integrins and most of cases showed normal or weakly depressed epithelial integrin expression (Fig. 8). Also, in contrast to E-cadherin, epithelial integrins did not demonstrate any tendency to be preserved more in tumors with weaker invasive potential. Strictly epithelial integrin subunits ($\alpha 2$, $\alpha 6$, $\beta 4$) expression correlated with each other ($R=0.48$), i.e. they trended to be preserved better in the same tumors.

3.3.2 Mesenchymal integrins

$\alpha 1$ integrin (collagen receptor) expressed stronger in advanced carcinomas, whereas $\alpha 5$ (fibronectin receptor) did not reveal such tendency (Table 3). However, both subunits expressed rather weakly (Fig. 8). In the whole UGC pool, $\alpha 1$ correlated with such signs of higher local invasive potential as bigger tumor size (AreaV, $R=0.51$), prevalence of extramucosal over mucosal portion ($R=0.50$), as well as with bigger number of LN metastasis ($R=0.39$). $\alpha 5$ integrin subunit did not demonstrate such linkage with invasion features, correlating only with bigger tumor size (AreaH, $R=0.57$) in advanced UGC.

Regarding prevailed histotype (SIG or POR) $\alpha 1$ and $\alpha 5$ demonstrated different tendencies. $\alpha 1$ in all UGC pool trended to express in mostly poorly differentiated tumors with smaller SRC and LS component ($R=0.53$) (Fig. 9A, poorly differentiated tumor [left pair] is positive; SRC tumor [right pair] is negative), whereas $\alpha 5$ in early UGC expressed stronger in SRC areas (Fig. 9B). In advanced UGC $\alpha 5$ demonstrated the same trend as $\alpha 1$, but it was non-significant. Both mesenchymal integrins expressed stronger in UGCs with prominent scirrhous component ($\alpha 1$ $R=0.50$, $\alpha 5$ $R=0.64$) (Fig. 9C).

3.3.3 αV group

Like mesenchymal integrin subunit, percentage of cells, expressing each individual integrin of this group was low, being significantly higher in advanced UGC only for $\alpha V\beta 5$ integrin (Table 3). αV subunit, which formed a part of all three integrins, as well as paired with two other β subunits ($\beta 1$, $\beta 8$), was expressed more often (Table 3). Tumors, expressing $\alpha V\beta 3$ and $\alpha V\beta 6$ integrins, expressed them in mostly in a weak manner (Fig. 8). Percentage of tumors with moderate or strong expression of $\alpha V\beta 5$ integrins and αV subunit was higher (Fig. 8).

In a whole tumor group αV integrin subunit expression demonstrated linkage with such local invasive features as bigger tumor size ($R=0.50$), prevalence of extramucosal (especially, mp) growth ($R=0.44$), as well as with γ -INF ($R=0.56$) and veins invasion ($R=0.37$). $\alpha V\beta 5$ integrin was also expressed more frequently if tumors with prevailed mp portion ($R=0.42$) and unclear invasion front (γ -INF, $R=0.37$). αV subunit was rarely seen in advanced cohesive UGC ($R=0.74$) (Fig. 10A) and advanced UGC with developed signet-ring cell ($R=0.53$) and mucinous component ($R=0.57$). Tubular component in advanced carcinomas was linked with stronger $\alpha V\beta 6$ expression ($R=0.51$) (Fig. 10B: only tubular, but not poorly differentiated component of the tumor expresses $\alpha V\beta 6$).

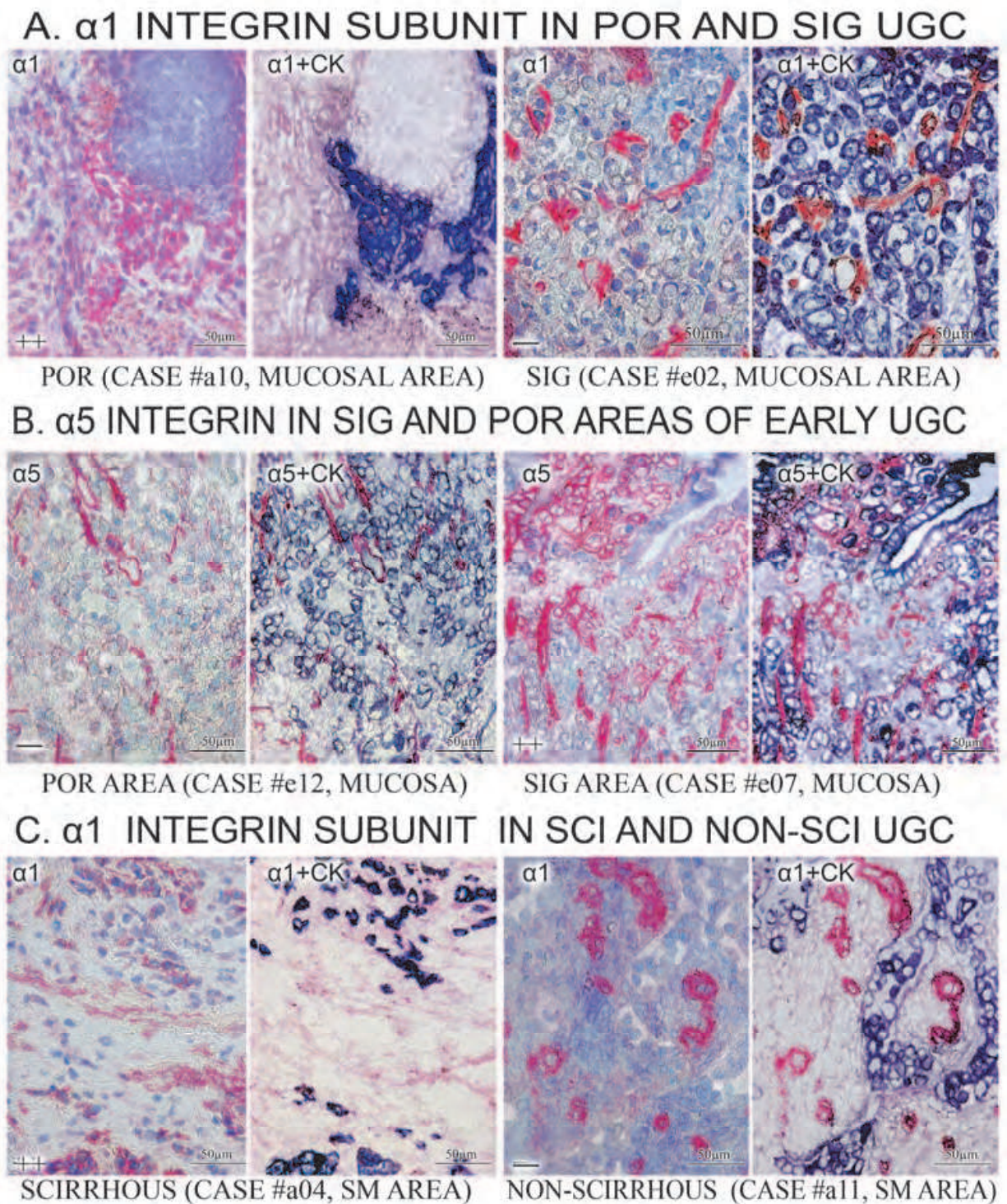


Fig. 9. Mesenchymal integrin expression. Microphotos of each area were taken twice: after staining for integrin (left photo in each pair; integrins are stained with magenta) and after staining for cytokeratins (2nd step of double staining, right photo, CK are stained with purple-blue). Double staining was performed for better quantitative assessment of integrin positive cells in frozen samples of UGC with poor morphological representation (for details see Yanchenko N. et al, 2009). Symbols in the bottom-left corner of each microphoto depict the intensity of integrin staining.

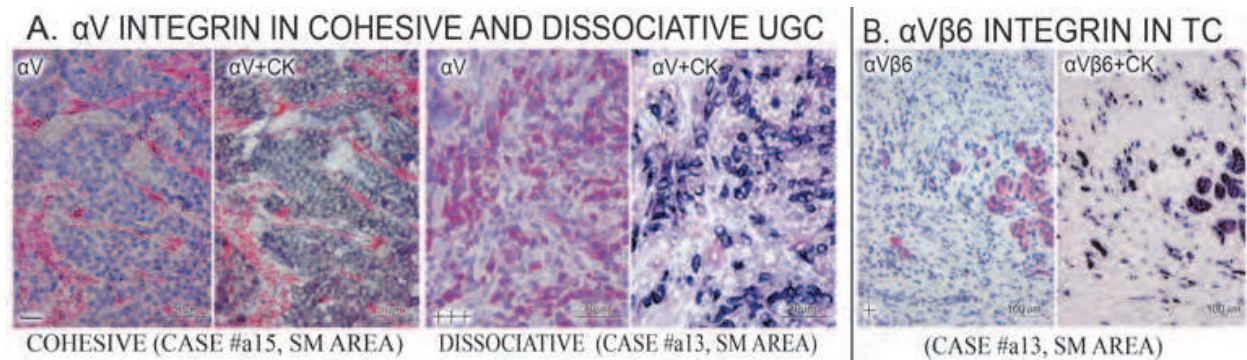


Fig. 10. αV -group integrin expression (for explanations see Fig. 9).

4. Discussion

4.1 Quantitative analysis proves loss of gastric differentiation and its impact into UGCs progression

Precise quantitative analysis provided plenty of data regarding UGCs structure and its impact into tumor progression. It quantitatively proved empirically observed tendency to lose gastric-cell differentiation while tumor invades in extramucosal areas, as we demonstrated substitution of relatively differentiated SRC component (in early carcinomas) by poorly differentiated component in advanced UGC.

Layered arrangement of SRC in mucosa, which reflects rudimentary gastric-mucosal differentiation (Natsagdorj et al., 2008; Sugihara et al., 1987) and is predominant in most early carcinomas, also demonstrated a tendency to be disorganised in advanced UGCs, and mucosal portions of those "primary" UGC which arose from LS-abundant early UGC almost totally lose this relatively ordered layered arrangement. Additionally, the more significantly advanced tumor loses LS, the more clearly it demonstrates the tendency for penetrative vs. spreading growth.

However, even those tiny remnants of LS are very important as they were shown to be necessary for prediction of the prognostic role of SRC component. We could conclude that the tendency for lymphatic (and venous) penetration, considered to be reason of poor prognosis in advanced SRC (Humar et al., 2007; Hyung et al., 2002; Kim et al., 1997; Li et al., 2007) is observed only in advanced "secondary" UGCs, arose from dedifferentiated tubular carcinomas, but not in advanced "primary" UGCs.

On the other hand, from the position of local invasive features prominent SRC component, in both early and advanced UGCs could be considered as favourable prognostic feature, as SRC demonstrated a tendency to spread in connective tissue (lamina propria in early UGCs or submucosa in advanced UGCs), but not penetrate muscle layers. We could suppose that one of the probable reasons observed in this study is lack of abnormal mesenchymal ($\alpha 1$ and $\alpha 5$) and αV -integrins (shown to be essential for mp penetration) in SRC.

Diffuse $\alpha 5$ integrin subunit expression in SRC areas of early carcinomas, was not associated with local invasive features and probably reflected its role in reparative (Herard et al., 1996) and inflammatory (Liebert et al., 1994) processes rather in invasion, as its ligand fibronectin is topographically associated with the front of invasion of GC (David et al., 1993).

4.2 E-cadherin loss in cohesive component of UGC

As could be expected, UGCs with predominantly cohesive cell arrangement and clearly seen invasion front demonstrated less aggressive behaviour. Surprisingly they also demonstrated

significantly higher tendency to lose E-cadherin expression than tumors with predominantly dissociative cell arrangement. This findings demonstrate that significance of solely E-cadherin down-regulation in cell dissociation could be rather questionable, especially in advanced UGC.

Simultaneously observed low expression of abnormal (mesenchymal and especially α V integrins) in highly cohesive tumors with compact, non-dissociative cell arrangement could mean that for dissociation and spreading UGC cells need not only to lose cell-cell adhesion but also to acquire abnormal cell-ECM contacts and motility mechanisms.

Previously, E-cadherin loss (extracellular domain was studied) was proved to be obligatory first step of carcinogenesis in hereditary diffuse GC, in sporadic early GC however such E-cadherin loss was considered as important, however optional event (Humar et al., 2007). Our data also proved that E-cadherin loss is not a critical requirement for tumor progression, as we demonstrated remarkable preservation of E-cadherin in both early and advanced sporadic UGC. Moreover, expression of E-cadherin in mucosal parts of advanced tumors with E-cadherin-negative extramucosal parts could mean that E-cadherin loss in such tumors is a secondary event, supporting the idea of gradual alteration of E-cadherin with UGC progression (Nakamura et al., 2005).

4.3 Scirrhous stromal component and mesenchymal integrin gain

Dissociative cell arrangement was clearly demonstrated to be not necessarily accompanied scirrhous stroma development, and even some cohesive tumors could develop areas of scirrhous stroma. However, in all cases scirrhous stroma prevalence contributed to local invasiveness. Possible reason of this findings is stronger expression of mesenchymal (α 1, α 5), but not epithelial (α 2, α V β 6) collagen and fibronectin receptors in scirrhous areas. It could be supposed that stronger desmoplastic response recruits integrins responsible for mesenchymal fibroblast-like movements, albeit fibronectin production is not directly linked to desmoplastic response (David et al., 1993).

Epithelial fibronectin receptor α V β 6 in the whole tumor was not correlated with scirrhous stroma development, as its expression was shown to be confined to invasion front (Breuss et al., 1995). However, α V β 6 association with tubular component in advanced UGC supports the idea of the its predominant role for cohort migration, which was proved to depend on fibronectin (Shimao et al., 1999).

4.4 E-cadherin down-regulation is more important than epithelial integrin down-regulation

In all UGCs studied normal epithelial integrin phenotype seems to be more stable than E-cadherin expression and did not demonstrate linkage with tumor invasiveness. One of the possible reasons of the absence of significant relationship between expression of normal integrin and tumor behavior is impossibility to predict their role in tumor spreading. They are characterised by broad range of modulation of their functions by external and internal signals, e.g. α 3 β 1 and α 6 β 4 integrins could form both sedentary and moving contacts, enhancing both anti- and pro-invasive properties of cancer cells (Giannelli et al., 2002). Conversely, E-cadherin is responsible only for homophilic adhesion and acting only as invasion suppressor, and its significant alteration causes an increase of local invasive features of UGC. However, as was mentioned above, even total loss of cell-cell adhesiveness without abnormal integrin gain does not lead to highly invasive phenotype.

From the position of the whole integrin phenotype, gain of abnormal integrins seems to be more important for UGC growth and invasion, than normal epithelial integrins down-regulation. Even minute or moderate gain of both mesenchymal and αV group integrins is linked with local invasion features such as tendency to penetrate deeper in muscularis propria, accompanied by γ infiltration pattern, linked with more rapid growth in UGC (our data) and poor prognosis of ovarian tumors (Spannuth et. al., 2005).

5. Conclusion

Thorough quantitated analysis of UGC histology, supplemented by simultaneous analysis of the integrin and E-cadherin phenotype, pioneered in this study, revealed not only tight correlation between UGCs histological components (such as signet-ring cell component, layer structures, cohesive cell arrangement, and scirrhous stromal development) and tumor invasive features, but also proposed E-cadherin down-regulation and abnormal (mesenchymal and αV -group) integrin up-regulation as the possible reason of this correlation.

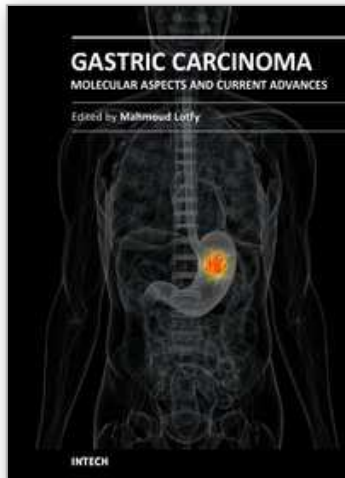
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Gastric cancer is one of the most common tumors worldwide. It has a heterogeneous milieu, where the genetic background, tumor immunology, oxidative stress, and microbial infections are key players in the multiple stages of tumorigenesis. These diverse factors are linked to the prognosis of the gastric cancer and the survival of gastric cancer patients. This book is appropriate for scientists and students in the field of oncology, gastroenterology, molecular biology, immunology, cell biology, biology, biochemistry, and pathology. This authoritative text carefully explains the fundamentals, providing a general overview of the principles followed by more detailed explanations of these recent topics efficiently. The topics presented herein contain the most recent knowledge in gastric cancer concerning the oncogenic signaling, genetic instability, the epigenetic aspect, molecular features and their clinical implications, miRNAs, integrin and E-cadherin, carbohydrate-associated-transferases, free radicals, immune cell responses, mucins, *Helicobacter-pylori*, neoadjuvant and adjuvant therapy, prophylactic strategy for peritoneal recurrence, and hepatic metastasis.

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