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Invited Review

Extracellular matrix in renal cell carcinomas

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Summary. Extracellular matrix (ECM) may be divided into interstitial matrix and the basement membrane (BM). ECM influences a variety of epithelial cell behaviours, including proliferation, differentiation, and morphogenesis, maybe most widely studied in kidney morphogenesis. In carcinomas, including renal cell carcinomas (RCCs), these properties and interactions of cells with interstitial matrix and BM are disturbed. As a carcinoma with a tendency to spread to distant sites, RCC is an interesting target for the study of epithelial-stromal interactions. Among interstitial collagens, type VI collagen appears to be widely distributed in RCCs. Also EDA-fibronectin (EDA-Fn) as well as tenascin-C (Tn) are important stromal components especially in poorly differentiated carcinomas. BMs of RCC islets and those of tumor blood vessel endothelia may merge in poorly differentiated carcinomas. As a dynamic component of BMs, laminins (Ln) are important in kidney development and RCC progression. Type IV collagen and nidogen, other components of BMs in RCCs, are produced by stromal as well as epithelial cells. ECM proteins may function in RCC progression by binding and regulating the activity of growth factors e.g. transforming growth factor ß1 and basic fibroblast growth factor. Also the expression of cell surface receptors for ECM is disturbed in RCCs. At least α_v integrin (Int) and CD44 emerge in renal epithelial cells during malignant transformation. Papillary renal neoplasms differ from RCCs by cell adhesion receptor expression and BM composition as well as by ECM avascularity and capacity to bind growth factors, thus suggesting a distinct property for this renal tumor.

Key words: Extracellular matrix, Basement membrane, Integrins, Growth factors

Introduction

Solid tumours are composed of two distinct compartments: the malignant parenchymal cells and the surrounding stroma. The stroma provides the vascular supply that tumours require to obtain nutrients, for gas exchange, and waste disposal (Yeo and Dvorak, 1995). The tumour stroma is composed of extracellular matrix (ECM) structural proteins, interstitial fluid as well as various cytokines and growth factors produced by stromal cells. The stroma contains endothelial cells, myofibroblasts, mast cells, histiocytes and variably inflammatory cells (Yeo and Dvorak, 1995).

ECM is an elaborate array of proteins and proteoglycans assisting in the organization of cells into complex organs. ECM components are assembled into various combinations, producing specific environments within tissues. ECM proteins are typically large glycoproteins such as fibronectin (Fn), interstitial collagens, elastin and tenascin (Tn) that assemble into fibrils or other complex macromolecular arrays (Gumbiner, 1996). ECM influences functions of the cells, and exerts its effects through a family of specific cell surface receptors. The most important receptors are integrins (Int), some cell surface proteoglycans and CD44 (Adams, 1997).

A specialized structure of ECM, the basement membrane (BM), is a thin sheet of proteins. BMs cover the basal surfaces of all epithelia (Merker, 1994) and are especially important in influencing epithelial cell polarity and differentiation, hence guiding the emergence of cellular phenotypes from embryonic development onwards (Timpl and Brown, 1996). BM is connected to cells by several cell surface receptors including Int family, which transduce signals from ECM to the cellular interior and vice versa (Mercurio, 1995; Adams, 1997). In the kidney glomerulus BM has a special function as a filtration barrier, in which the laminin (Ln) ß2 chain appears to play an important role (Noakes et al., 1995). In addition to the aforementioned functions of BM, it serves as a structural barrier between tissue compartments, for instance in carcinomas, by separating the parenchymal cells from the surrounding stromal compartment. In epithelia, BM is formed in cooperation by epithelial and mesenchymal cells (Timpl and Brown, 1996).

In carcinomas, the epithelial-stromal interactions undergo changes that alter the regulation of the growth and function of cells, tissues and organs. Stroma-derived factors and interactions between ECM and neoplastic cells play a role in tumour cell migration as well as

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proliferation (Yeo and Dvorak, 1995; Wernert, 1997). The importance of tissue architecture is suggested by recent results on breast carcinoma cells which demonstrate that as long as the tissue architecture is maintained, the phenotype can override the genotype (Weaver et al., 1997).

In this review, we will discuss the composition and significance of ECM in renal cell carcinomas (RCCs).

Histology of renal cell carcinomas

Five histological types of renal carcinomas have been distinguished: clear-cell, chromophilic (papillary), chromophobic, oncocytic, and collecting-duct (Bellini's duct) tumours (for a review, see Weiss et al., 1995). Clear-cell carcinomas are the most common (75 to 85 percent) and the most widely studied renal tumours (Motzer et al., 1996). Further on in this review, we will use the term renal cell carcinoma (RCC) as a synonym for clear cell type RCC.

RCC is defined as a tumour composed of mixtures of cells with clear and granular cytoplasm arranged in non-papillary formations. Parenchymal cells of RCCs are most often organized into sheets or broad trabeculae, separated by a richly vascular fibrous stroma (solid pattern), or arranged around central spaces (glandular pattern) or lining cysts. The proliferating cells may be spindle-shaped and in high-grade tumors may acquire a sarcomatoid appearance (Weiss et al., 1995).

Interstitial matrix of renal cell carcinomas

Many different types of collagens have been described, all composed of distinct α chains. Among interstitial collagens, type I collagen is most often assumed to be the cause of pathological fibrosis, but in the kidney it is rather deposited in minute amounts late in the fibrosis (Furness, 1996). A much more abundant component of fibrotic matrix in the kidney is type III collagen (Furness, 1996). In RCCs collagen types I and III were detected in stroma of ca. half of the specimens by Droz et al. (1994). Also type VI collagen is a component of the ECM of normal human kidney (Magro et al., 1996). The expression of type VI collagen undergoes changes during kidney development, and in the studies of Magro et al. (1996) it has been located in mature kidney to mesangium, intertubular interstitium, renal capsule and to the BMs of Bowman's capsule, tubules and collecting ducts and to a lesser extent to the BM of glomeruli. However, our immunofluorescence results differ distinctly from those of Magro et al. (1996), suggesting that type VI collagen is clearly expressed in BMs of glomeruli (Fig. 1A), and, on the other hand, we did not find it in the BMs of tubules and collecting ducts (Fig. 1B) (Footnote, Lohi et al., unpuplished results). The difference in results may be due to a greater resolution of the immunofluorescence method used by us, in comparison to avidin-biotin-peroxidase complex technique used by Magro et al., (1996). Type VI collagen has been suggested to act as an anchoring component linking the epithelial BMs with the ECM in developing mesonephric structures (Magro et al., 1995), hence resembling in function type VII collagen in stratified and compound epithelia (Wetzels et al., 1991). The distribution of type VI collagen in RCCs has not been described in the literature. Our immunofluorescence staining results suggest that type VI collagen is widely distributed in the stroma of RCCs (Fig. 1C) and occasionally appears to be present also in BM of RCC cell nests (Fig. 1D).

Among non-collagenous ECM glycoproteins in carcinomas a special interest has been devoted to Fn and Tn-C. They are modular proteins produced as several isoforms by differential splicing of mRNA (Chiquet-Ehrismann, 1995; Ffrench-Constant, 1995).

Fn is an extensively studied ECM glycoprotein. Originally it was characterized as a glycoprotein lost upon transformation (Vaheri and Ruoslahti, 1974; Hynes et al., 1978; Vaheri and Mosher, 1978). Knock-out mice lacking Fn die during embryonic development (George et al., 1993). Cellular adhesion has been attributed to a major biological function of Fn. Fn may also be a potential regulator in cancer cell growth. A truncated 178 kD fragment of Fn has been suggested to function as an autologous growth-promoting substance in RCC cell cultures (Kochevar et al., 1992), On the other hand, an RCC cell line was stimulated to migrate in response to Fn (Grossi et al., 1992) and a chemotactic ability for Fn was suggested to correlate with the metastatic potential of cultured RCC cells (Murata et al., 1992).

The alternative splicing of Fn gene product produces two major Fn isoforms, named extradomain-A (EDA) and EDB Fns. An additional isoform is produced by differential glycosylation of the variable region and has been named as oncofetal Fn (Matsuura et al., 1989). In normal human renal tissue EDA-Fn is only scarcely expressed in endothelia of larger vessels and in the mesangial matrix (Laitinen et al., 1991), but its expression is greatly increased in diverse variants of inflammatory glomerular disease as well as in chronic rejection (Gould et al., 1992; Assad et al., 1993). EDB-Fn and oncofetal-Fn are expressed in developing kidney, but are absent in normal adult kidney (Laitinen et al., 1991). Their expression is upregulated in association with cellular proliferation and/or necrosis in pathological conditions of human glomeruli (Assad et al., 1993). EDA-Fn is widely distributed in the stroma of RCCs, while the other isoforms are expressed only scarcely (Lohi et al., 1995), but they are abundant in stromal tissue of some other carcinomas (Kaczmarek et al., 1994; Koukoulis et al., 1995). However, we have found all the aforementioned Fn isoforms in xenograft tumours of RCC cells, suggesting that stromal factors are responsible for the production of various Fn isoforms (Lohi et al., 1995).

Tn-C is a disulfide-linked hexamer expressed in many developing organs at sites of epithelial-mesenchymal interaction (Chiquet-Ehrismann, 1995). Tn-C is

present in a restricted pattern in mature tissues, but appears in diverse reactive conditions (Koukoulis et al., 1991) and in the stroma of various carcinomas (see, e.g. Howeedy et al., 1990; Koukoulis et al., 1991; Natali et al., 1991; Soini et al., 1992; Ibrahim et al., 1993; Tiitta et al., 1993, 1994). Stromal Tn expression in carcinomas has often been correlated with the degree of inflammation present in the tissue (Natali et al., 1991; Tiitta et al., 1992, 1993; Moch et al., 1993). In the usual interstitial pneumonia it has been suggested to be a marker of poor prognosis (Kaarteenaho-Wiik et al., 1996).

The significance of Tn as a prognostic marker in carcinomas is under an intense study in several laboratories. Our studies have shown that the expression of Tn in the invasion border of early breast cancer correlates with a high risk of distant metastasis (Jahkola et al., 1996). On the other hand, the results of Shoji et al. (1993) suggested that the local production of Tn by carcinoma cells might be potential marker of favorable prognosis. The biological function of Tn-C has been under discussion during recent years. Various roles for Tn-C in directing morphogenesis or functioning as anti-adhesive molecule or mitogen have been suggested (Erickson, 1993; Chiquet-Ehrismann, 1995). On the other hand, Saga et al. (1992) reported, based on Tn-C gene knockout experiments, that «mice develop normally without Tn».

In normal human kidney Tn-C is expressed strongly in the mesangial matrix, weakly in medullary and tubular interstitium and variably in Bowman's capsular area (Koukoulis et al., 1991; Gould et al., 1992; Truong et al., 1994, 1996). The expression of Tn-C is increased in inflammatory glomerular disease as well as in chronic rejection (Gould et al., 1992; Assad et al., 1993). In well differentiated RCCs, Tn-C is located to the BM zone and around blood vessels, whereas in poorly differentiated carcinomas Tn-C is widely distributed throughout the stromal compartment (Lohi et al., 1995). Interestingly,

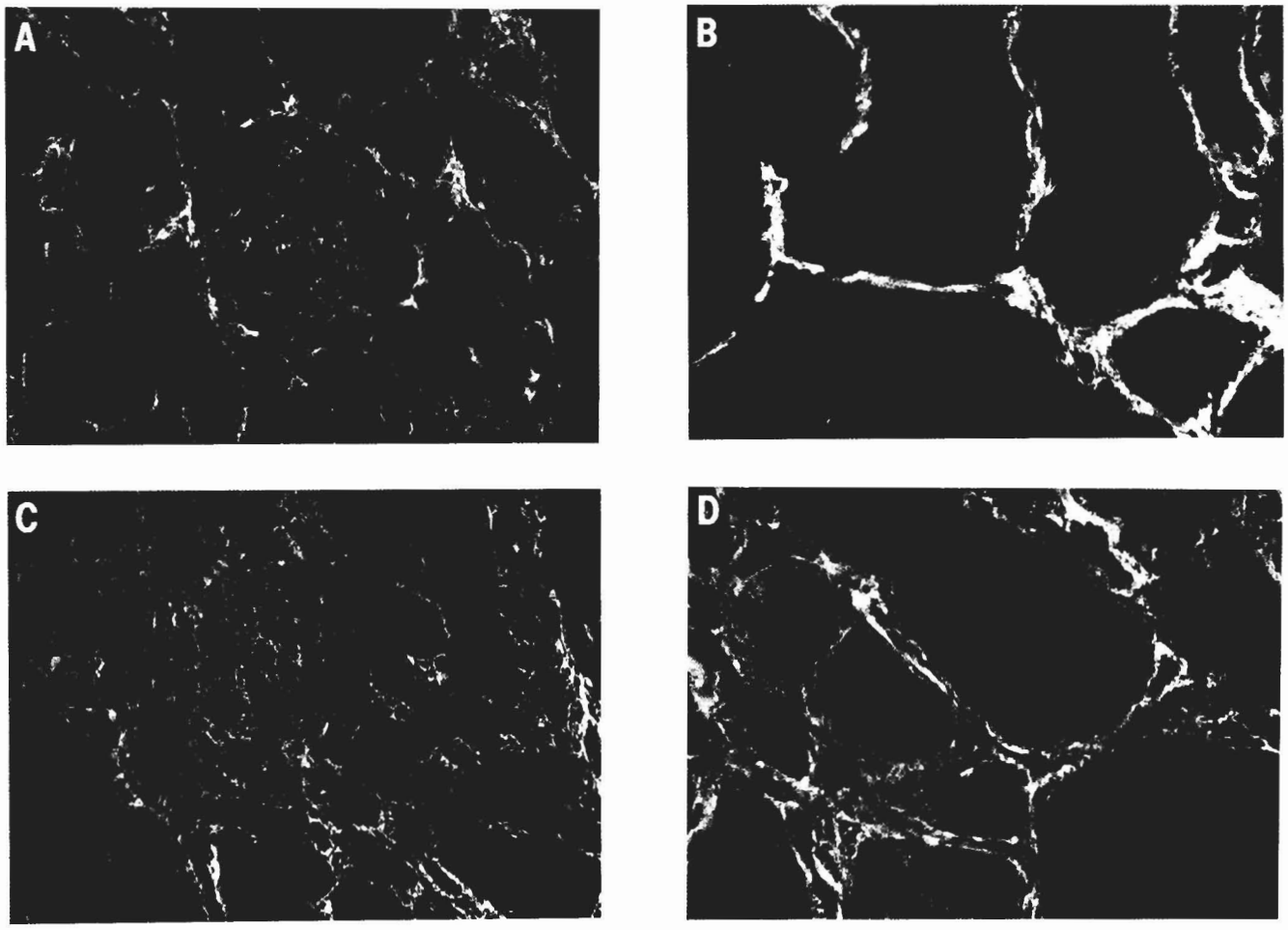

Fig. 1. Immunoreactivity for type VI collagen is detectable in BM of glomerulus and in the tubular area of adult human kidney **(A)**. The figure with the higher magnification clearly demonstrates that immunoreactivity for type VI collagen in tubular area of kidney is located to the interstitial matrix, but not to the BMs **(B)**. A widely distributed immunoreactivity for type VI collagen is present in the stromal compartment of poorly differentiated (gradus 3, WHO) RCC **(C)**. With higher magnification, immunoreactivity for type VI collagen is detectable in the interstitial matrix between carcinoma cell islets and occasionally also in the BMs of well differentiated RCC (gradus 1, WHO) **(D)**. A, B, x 200; C, D, x 380

we have found that in the experimental xenograft tumours derived from RCC cells, Tn-C distribution is associated with the grade of differentiation of the tumours in the same way as in RCCs (Lohi et al., 1995). Among the two distinct isoforms of Tn-C (Erickson, 1993), the low molecular weight isoform appears to be predominant in RCCs (Lohi et al., 1995), while both isoforms are prominent in breast carcinomas (Howeedy et al., 1990).

Another protein of the Tn family, Tn-X, has been found in culture medium of renal carcinoma cells (Matsumoto et al., 1994). Tn-X is prominent in skeletal and heart muscle (Chiquet-Ehrismann, 1995), but as with Tn-C its functions remain to be elucidated. Our results suggest that unlike Tn-C, Tn-X is scarcely expressed in fetal tissues but emerges during maturation. In kidney, Tn-X is variably found in the interstitium and Bowman's capsule, but not in the mesangial matrix. RCCs appeared to be rather negative for Tn-X.

Basement membranes in renal cell carcinomas

BM discontinuities have generally been associated with malignancy in various types of carcinomas (for original studies see Albrechtsen et al., 1981; Barsky et al., 1983; for reviews, see Bosman, 1994; Flug and Köpf-Maier, 1995). Liotta and Stetler-Stevenson (1991) have proposed a three step theory for the invasion of carcinoma cells through BMs. The steps include: tumour cell attachment to the ECM, proteolysis of BM and tumour cell locomotion. On the other hand, both malignant invasive tumours with continuous BM as well as noninvasive tumours with highly defective BM have been described (See for rewiews, Bosman, 1994; Flugh and Köpf-Maier, 1995). In RCCs, discontinuity of BM is associated with a high grade of differentiation (Korhonen et al., 1992b).

Type IV collagen together with Ln forms the structural framework of BM. Type IV collagen is a trimeric protein and the most widely expressed type IV collagen trimer is composed of two α1(IV) chains and one α2(IV) chain (Hudson et al., 1993). The α3(IV)-α6(IV) chains have a more restricted tissue distribution, but are expressed in kidney. In the normal adult human kidney, the α3(IV)-α6(IV) chains were distributed to the Bowman's capsular BMs, distal tubular or collecting duct BMs and α3(IV)-α5(IV) chains additionally to the glomerular BM (Miner and Sanes, 1994; Yoshioka et al., 1994; Mårtensson et al., 1995; Ninomiya et al., 1995; Peissel et al., 1995; Lohi et al., 1997a).

The expression of collagen $\alpha 2_2\alpha 1$(IV) trimer in BMs of RCCs has been described in several studies (Korhonen et al., 1992b; Droz et al., 1994; Mårtensson et al., 1995; Lohi et al., 1997a). Interestingly, trimers containing collagen α3(IV)-α6(IV) chains are also present in most of the RCCs (Lohi et al., 1997a). Both RCC cells as well as stromal cells appear to be potential sources for production of collagen α1(IV) and α2(IV) chains as recently suggested by us based on xenograft experiments using species-specific antibodies (Lohi et al., 1997a). Collagen α3(IV)-α6(IV) chains were, however, not expressed in RCC-derived xenograft tumours (Lohi et al., 1997a).

Lns are trimeric BM proteins composed of three different polypeptide chains. The first Ln trimer was isolated from a mouse tumour, Engelbreth-Holm-Swarm tumour, from which the name EHS-Ln is derived (Chung et al., 1979; Timpl et al., 1979). Later, after more Ln trimers had been isolated, EHS-Ln was renamed Ln-1 (Burgeson et al., 1994). Presently, five different α chains as well as three ß and three γ chains have been described and they are presumed to form at least 11 distinct trimers named Lns-1 to -11 (Burgeson et al., 1994; Timpl and Brown, 1996; Miner et al., 1997).

Lns are responsible for many BM functions; for instance, the establishment of epithelial cell polarity and induction of epithelial cell differentiation during development (Adams and Watt, 1993; Timpl and Brown 1996). The significance of Ln in epithelial cell polarization during morphogenesis was initially suggested in an *in vitro* tubulogenesis model by Klein et al. (1988) and its significance in tubule formation was implicated by antibody inhibition experiments in organ cultures by Sorokin et al. (1992). Lns have a tissue-specific distribution, and this distribution varies during development in various tissues (Klein et al., 1990; Adams and Watt, 1993; Engvall, 1993; Lohi et al., 1996a, 1997b; Virtanen et al., 1996, 1997). Changes in the expression of Ln chains during nephrogenesis have been studied especially widely. For example, in the glomerular BM Ln ß2 chain replaces Ln ß1 chain during development of human, mouse and rat nephron (Virtanen et al., 1995; Durbeej et al., 1996; Miner et al., 1997). Among different Ln α chains, loss of α4 and further loss of Ln α1 chain in glomerular BM during mouse nephrogenesis has been reported recently (Miner et al., 1997). Hence, Ln α5 chain is the only Ln α chain present in mature mouse glomerular BM (Miner et al., 1997). Interestingly, the developmental transition in Ln α and ß chains has been suggested to be regulated independently of each other (Miner et al., 1997). Summarizing the variable expression of various Ln chains during mouse nephrogenesis, Miner et al. (1997) have suggested that Ln-1 (α1ß1γ1), -3 (α1ß2γ1), -8 (α4ß1γ1), -9 (α4ß2γ1), -10 (α5ß1γ1), and -11 (α5ß2γ1) trimers may be present transiently in developing glomerular BM. Among different mouse tubular segments, α1 is present primarily in proximal tubular BMs, α2 in a subset of corticomedullary tubular BMs, and α5 in all BMs (Miner et al., 1997). In human kidney, Ln-1 is present in all tubular BMs of adult human kidney (Virtanen et al., 1995), whereas Ln-5 is located to a thin segment of the loop of Henle (Lohi et al., 1996b). RCCs express at least Ln α1, ß1, ß2 and γ1 chains in their BMs (Lohi et al., 1996b). RCCs variably produce different Ln chains in cell culture conditions as well as in xenograft tumours, and the regulation is at least partially realized by stromal factors (Lohi et al., 1996b).

Our recent immunostaining experiments for Ln and type IV collagen, both major BM components, have shown that occasionally in poorly differentiated RCCs it is not possible to differentiate BM structures of carcinoma cells from those of endothelial cells by resolution of the immunofluorescence microscope. Therefore, we have proposed that a merging of BMs of RCC cells and endothelial cells takes place in poorly differentiated RCCs (Lohi et al., 1996b, 1997a). In this respect, it is of interest that the RCCs have a tendency to spread by hematogenous route (Motzer et al., 1996). Hyaline globules (extracellular collections of amorphous material) in RCCs have recently been suggested to contain multilayered accumulation of BM material, Ln and type IV collagen (Gatalica et al., 1997).

Nidogen is a BM glycoprotein consisting of three globular domains (Timpl et al., 1983; Paulsson et al., 1986; Dziadek, 1995). Nidogen binds Ln to type IV collagen (Timpl and Brown, 1996). Nidogen has been demonstrated to be widely present in the various BMs of human kidney (Katz et al., 1991), but there are no reports describing the expression of nidogen in RCCs. Nidogen is suggested to be derived from mesenchymal cells only (Ekblom et al., 1994; Thomas and Dziadek, 1993). Our unpublished results suggest that some RCC cell lines are able to produce nidogen in culture conditions and in xenografts of nude mice (Oivula et al., in preparation). A basic structural proteoglycan of the BMs, heparan sulfate proteoglycan, is known to be present in the BMs of the RCCs (Droz et al., 1994).

Cell surface receptors for extracellular matrix in renal cell carcinomas

Perhaps the most important receptors for ECM proteins are Ints, which are transmembrane glycoproteins consisting of one α and one ß subunit (Ruoslahti, 1991; Hynes, 1992). At least 16 α and 8 ß subunits are known to exist (Streit et al., 1996; Varner and Cheresh, 1996). Ints are believed to play a role e.g. in signal transduction, gene expression, proliferation, apoptosis regulation, embryogenesis, inflammation, tumour progression and metastasis (Albelda and Buck, 1990; Schwartz et al., 1995; Varner and Cheresh, 1996). Although a wealth of changes in the expression of Ints in malignant transformation has been reported (Streit et al., 1996), it is notable that there do not appear to be any general changes found in different types of malignancies, or shared by, for instance, most carcinomas.

Different Ints are characteristically expressed in specific nephron segments in the human kidney. Among α subunits of Ints, α_2 Int subunit is expressed in distal tubules and collecting ducts, whereas α_3 subunit was located in distal tubules as well as glomerular podocytes. α_6 subunit is widely present in all tubules (Korhonen et al., 1990b). Among ß Ints, $ß_3$ Int has a more restricted distribution than $ß_1$ Int (Korhonen et al., 1990a). $\alpha_2ß_1$, $\alpha_3ß_1$ and $\alpha_6ß_1$ Int complexes are suggested to be involved in glomerulogenesis, whereas the $\alpha_5ß_1$ and $\alpha_4ß_1$ may only play minor roles (Korhonen et al., 1990b). An important role for α_3 Int but not for Int α_6 in branching morphogenesis in kidney development was recently shown by gene knockout experiments by Kreidberg et al. (1996) and Georges-Labouesse et al. (1996). These are results in some ways contradictory to antibody inhibition studies of Sorokin et al. (1990).

RCC cells are known to variably express among ß1 integrins α_3, α_4, α_5, and α_6 Int subunits (Korhonen et al., 1992b; Droz et al., 1994; Tomita et al., 1995; Gilcrease et al., 1996; Rabb et al., 1996). Results from our laboratory suggested that a decreased expression of int α_6 subunits would correlate with increasing histological grade of human RCCs (Korhonen et al., 1992b), but this suggestion has later been challenged (Terpe et al., 1993; Droz et al., 1994). Int $\alpha_4ß_1$ is not detectable in adult human kidney (Cosio et al., 1990; Korhonen et al., 1990b, 1992a; Simon and Mc Donald, 1990; Adler, 1992), but is transiently expressed in the uninduced, not yet condensed mesenchymal cells during early development (Korhonen et al., 1992a). However, it has been suggested that it is expressed in most of the metastatic RCCs (Tomita et al., 1995; Gilcrease et al., 1996). Furthermore, adhesion of RCC cells to human umbilical-vein endothelial cells was inhibited by antibodies to Int α_4 (or vascular cell adhesion molecule-1), suggesting that Int $\alpha_4ß_1$ complex might play a role in the hematogenous spread of RCCs (Tomita et al., 1995). Int $\alpha_5ß_1$ complex, which has been described to be weakly present on endothelial and mesangial cells in the normal kidney (Cosio et al., 1990; Adler, 1992) or totally absent as detected with several antibodies (Korhonen et al., 1992b), has been associated with metastatic RCCs or extrarenal invasion (Gilcrease et al., 1996).

A specially important biological role has been suggested for α_v Int subunit. α_v Int associates with multiple ß subunits forming distinct heterotrimers. $\alpha_vß_3$ Int has been suggested to influence cellular proliferation (Varner and Cheresh, 1996). In the normal kidney α_v Int is localized to the glomeruli, Bowman's capsule and vascular endothelium (Patey et al., 1994; Rabb et al., 1996). Int $\alpha_vß_5$ is also located in glomerular epithelial cells and occasionally to Bowman's capsule. Focal expression has also been claimed to be present in tubular epithelial cells (Patey et al., 1994; Rabb et al., 1996). An increased expression of α_v Ints has often been correlated with increasing malignancy (Varner and Cheresh, 1996). The α_v Int is expressed in RCCs, but there is no agreement as to whether it is associated with the grade of malignancy (Korhonen et al., 1992b; Droz et al., 1994; Rabb et al., 1996).

CD 44 is a widely expressed cell surface glycoprotein with functions e.g. in lymphocyte homing and activation, hematopoiesis, cell migration, binding of certain cytokines to the endothelium, and tumour metastasis (Streit et al., 1996). The epithelial isoform of CD44 mediates cell-extracellular matrix interactions by

recognizing hyaluronic acid (Culty et al., 1990; Underhill, 1992). CD44 has been found to be lacking in tubular and glomerular epithelial cells in the human kidney and to be expressed only in the interstitial tissue (Gilcrease et al., 1996). However, part of the RCCs are known to express CD44 (Terpe et al., 1993; Gilcrease et al., 1996). The expression of CD44 has been associated with extrarenal invasion or with known metastases at the time of nephrectomy (Gilcrease et al., 1996), but does not show any correlation with RCC tumour grade (Terpe et al., 1993). On the other hand, clear cell type RCCs differ from chromophilic cell type variants by their expression of CD44 (Heider et al., 1996).

On the origin of renal cell carcinomas

The origin of RCC has been under continuous debate. Based on immunohistochemical studies on various cytoplasmic or cell membrane antigens both proximal (Holthöfer et al., 1983; Borowitz et al., 1986; Gröne et al., 1986; Oosterwijk et al., 1986, 1990) and distal (Fleming et al., 1985; Blouin et al., 1989; Korhonen et al., 1992b) tubular origins have been proposed for RCCs. Recently, BM components typical for distal tubular BM were found in RCCs (Mårtensson et al., 1995; Lohi et al., 1997a). However, the expression of various cellular or extracellular proteins in carcinomas may not necessarily reflect the properties of their cell of origin.

Papillary renal neoplasms were traditionally considered to be histological variants of the usual renal adenocarcinomas, despite characteristic features of this subtype. Recently, papillary neoplasms were found to have cytogenetic abnormalities markedly different from other renal adenocarcinomas, justifying their consideration as a distinct type of neoplasms (Kovacs, 1989). In line with cytogenetically distinctive features, we have described a distinctive BM composition in papillary renal neoplasms. In distinction from non-papillary RCCs, papillary renal neoplasms express Ln-5 and on the other hand lack α3, α4 and α6 collagen IV chains in their BMs (Lohi et al., 1996b, 1997a). The expression of Ln-5 mimics characteristics of the thin segment of the loop of Henle (Lohi et al., 1996b). However, it is more evident that papillary renal neoplasms are derived from collecting ducts as was suggested earlier based on morphological data (Mancilla-Jiménez et al., 1976). Ln-5 is a ligand for both Int $\alpha_6\beta_4$ (Borradori and Sonnenberg, 1996; Niessen et al., 1994) and $\alpha_3\beta_1$ (Carter et al., 1991; Zhang and Kramer, 1996). Interestingly, papillary renal neoplasms unlike RCCs are also immunoreactive for α_6 (Fig. 2A) and β_4 Int subunits (Fig. 2B) and one of our two tumor specimens was also positive for hemidesmosomal antigen-1 (data not shown). BMs of papillary renal neoplasms also differ from RCCs by their binding of fibroblast growth factor-7 (Friedl et al., 1997). Another interesting difference in papillary renal neoplasms in comparison to RCCs is their avascularity (Mancilla-Jiménez et al., 1976; Boczko et al., 1979). Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) is a multifunctional cytokine playing a role e.g. in angiogenesis by stimulating endothelial cell proliferation (Senger et al., 1993). VPF mRNA has been reported to be lacking in papillary renal neoplasms, although it has been found in RCCs (Brown et al., 1993).

Spreading of renal cell carcinomas

RCC tends to invade the renal vein and the inferior vena cava, occasionally reaching the right atrium (Motzer et al., 1996). The cellular and molecular mechanisms functioning in RCC spread have been widely investigated. Based on experiments in three dimensional matrigel culture by Yang et al. (1990), RCC cells have been suggested to reorganize ECM in their immediate vicinity. High nuclear grade carcinoma cells have been suggested to be the most invasive in matrigel cultures (Yang et al., 1990).

Cytokines are important in the homing and migra-

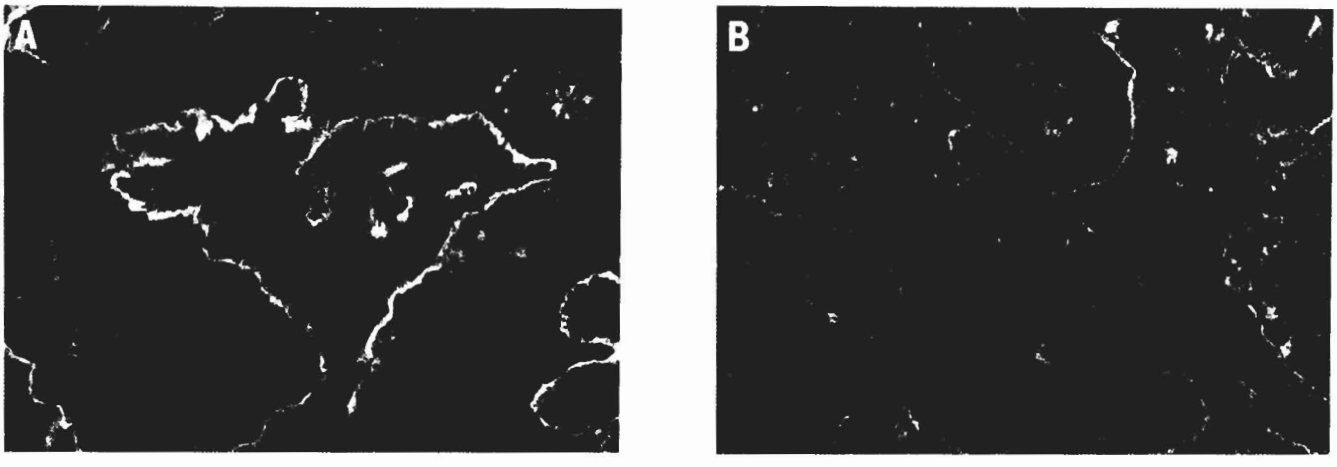

Fig. 2. A polarized immunoreactivity for α_6 **(A)** and β_4 **(B)** integrins was detectable in the papillae of papillary renal neoplasm. x 300

tion of lymphocytes and adhesion between leukocytes and endothelium (Chin et al., 1990). Similar mechanisms may operate in the spread of malignant cells. *In vitro* invasion assay experiments by Yanase et al., (1995) have shown that tumour necrosis factor, interleukin-1 and interleukin-6 increase the invasiveness of RCC cells by assisting their attachment to endothelia. Treatment with the aforementioned cytokines increases the expression of vascular cell adhesion molecule-1 in endothelial cells, but does not affect type IV collagenolysis (Yanase et al., 1995). On the other hand, the invasiveness of RCC cells is affected by MAb to vascular cell adhesion molecule-1. The interaction of $\alpha_4\beta_1$ Int complex on the surface of the RCC cells with vascular cell-adhesion molecule-1 (VCAM-1) in endothelial cells is a potential candidate for a mechanism operating in hematogenous metastasis of RCCs, as discussed earlier in this review (Tomita et al., 1995). Interleukin-2 has been suggested to modify the expression of intercellular adhesion molecule-1 on leukemia blasts (Olive et al., 1991). Interleukin-2-transfected RCC cells are reported to have a decreased binding affinity to Fn, Ln, type IV collagen and vitronectin (Hathorn et al., 1994). Hence, the local production of high concentrations of interleukin-2 and interferon-α at the tumour site may directly alter the interaction between RCC cells and ECM as well as the invasive and metastatic phenotype of the tumor (Hathorn et al., 1994). On the other hand, αIFN and γIFN have an antiproliferative effect for cultured RCC cells (Buszello, 1995).

The interaction between epithelial and mesenchymal cells is important for epithelial differentiation (see e.g. Fritsch et al., 1997; Plateroti et al., 1997). The mesenchymal fibroblasts also play an important role in various pathological conditions: e.g. in wound healing and fibrocontractive diseases (Desmoulière and Gabbiani, 1996). They have also been involved in invasion and metastatic process: e.g. in colorectal carcinomas (Martin et al., 1996). Experiments with RCC cells have suggested that kidney -derived fibroblasts regulate their production of degradative enzymes (Gohji et al., 1994). Hence, fibroblasts may influence the invasive and metastatic capacity of RCC cells (Gohji et al., 1994).

ECM or BM proteins may also function in RCC spread also by binding distinct cytokines e.g. growth factors. The regulation of the activity of growth factors is mostly based on their binding to ECM, and rapid extracellular signaling is generated by proteolytic release and activation of stored growth factors (Taipale and Keski-Oja, 1997). For examble, basic fibroblast growth factor has been suggested to interact with ECM and BM (Folkman, 1988; Flaumenhaft et al., 1989). Basic fibroblast growth factor is a pleiotropic growth factor, which is important in kidney development (Karavanova et al., 1996). Basic fibroblast growth factor has been suggested to play a role in growth and metastasis of various tumours (Nguyen et al., 1994). It is also a potent regulator in RCC spread (Duensing et al., 1995). Cytoplasmic expression of fibroblast growth factor has been suggested to be a marker of poor prognosis in RCC (Nanus et al., 1993) and increased serum basic fibroblast growth factor levels have been reported to be associated with a higher frequency of progressive pulmonary metastases (Duensing et al., 1995).

Hepatocyte growth factor/scatter factor is a multifunctional effector of cells expressing c-met receptor in their cell membrane (Jeffers et al., 1996). Hepatocyte growth factor is involved in various cellular and tissue processes including kidney development (Santos et al., 1994; Woolf et al., 1995). The c-met/hepatocyte growth factor receptor has been suggested to be overexpressed in the RCCs. It is also overexpressed in the RCC cell line, the motility of which is trigged by HGF in invasion chamber model (Natali et al., 1996). Sulfoglycolipids on RCC cells might function as reservoirs for HGF (Kobayashi et al., 1994a). HGF stimulates the proliferation and motility of RCC cells, suggesting that HGF has multiple biological activities in RCC cells (Kobayashi et al., 1994b).

Transforming growth factor ß is secreted from cells in latent form with propeptide, and it is activated later (Taipale and Keski-Oja, 1997). Transforming growth factor ß is thought to associate with ECM and BM and its active form is suggested to be bound e.g. to type IV collagen and fibronectin (Taipale and Keski-Oja, 1997). Transforming growth factor ß1 has been considered as G1 phase-arresting inhibitory growth regulator for epithelial cells (Reddy et al., 1994). Transforming growth factor was suggested to suppress growth of two RCC cell lines *in vitro* (Wade et al., 1992). In the later experiments with 30 RCC cell lines, Ramp et al. (1997) found transforming growth factor ß1-resistant cells, and concluded that transforming growth factor ß1 resistance is an important factor for tumor progression in RCC.

Extracellular matrix remodelling

Some proteolytic remodelling of the ECM inevitably takes place during malignant progression of carcinomas (Liotta and Stetler-Stevenson, 1990), and among proteases, the matrix metalloproteases have been shown to present an altered distribution and activity in malignant tissues (Coussens and Werb, 1996). One of the functions for endogenous growth factors produced by carcinoma cells could be to induce the invasiveness or metastatic potential of cancer by increasing the production of degradative enzymes. Epidermal growth factor, which has a wide spectrum of activities including mitogenic, chemotactic and angiogenic effects (Khazaie et al., 1993) was reported to modulate the *in vitro* invasion, motility, and adhesiveness of RCC cells (Price et al., 1996). Additionally, epidermal growth factor increases their production of 92 kDa metalloproteinase (MMP-9; Price et al., 1996). On the other hand, overexpression of endogenous native fibroblast growth factor-2 has been suggested to play a role in the invasion and metastasis of renal cell carcinoma, through the production of MMP-2 (Miyake et al., 1996).

Gelatinase (MMP-2) production by RCC cells is influenced by organ microenvironment. Basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor $ß_1$ appear to stimulate gelatinase expression by the cultured RCC cells. Kidney fibroblasts regulate by production of transforming growth factor ß1 the production of degradative enzymes by RCC cells (Gohji et al., 1994).

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