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Mammalian Collagen IV

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Abstract

Four decades have passed since the first discovery of collagen IV by Kefalides in 1966. Since then collagen IV has been investigated extensively by a large number of research laboratories around the world. Advances in molecular genetics have resulted in identification of six evolutionary related mammalian genes encoding six different polypeptide chains of collagen IV. The genes are differentially expressed during the embryonic development, providing different tissues with specific collagen IV networks each having unique biochemical properties. Newly translated α -chains interact and assemble in the endoplasmic reticulum in a chain-specific fashion and form unique heterotrimers. Unlike most collagens, type IV collagen is an exclusive member of the basement membranes and through a complex inter- and intramolecular interactions form supramolecular networks that influence cell adhesion, migration, and differentiation. Collagen IV is directly involved in a number of genetic and acquired disease such as Alport's and Goodpasture's syndromes. Recent discoveries have also highlighted a new and direct role for collagen IV in the development of rare genetic diseases such as cerebral hemorrhage and porencephaly in infants and hemorrhagic stroke in adults. Years of intensive investigations have resulted in a vast body of information about the structure, function, and biology of collagen IV. In this review article, we will summarize essential findings on the structural and functional relationships of different collagen IV chains and their roles in health and disease.

Keywords

collagen IV; basement membrane; Alport's syndrome; Goodpasture's syndrome

Introduction

Type IV collagen is a unique member of the large collagen superfamily which in vertebrates comprise 28 different types (Myllyharju and Kivirikko, 2004; Veit et al., 2006). Unlike most collagens, type IV collagen occurs only in the basement membranes (BMs) and comprises up to six genetically distinct α -chains designated $\alpha 1(\text{IV})$ to $\alpha 6(\text{IV})$. Out of many potential combinations, the chains interact and assemble with a remarkable specificity to form only three distinct heterotrimers of $\alpha 1\alpha 1\alpha 2$, $\alpha 3\alpha 4\alpha 5$, and $\alpha 5\alpha 5\alpha 6$. The $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ chains, which were first to be described and thus called “classical” chains, are present in the

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BM of all tissues, whereas the other four chains have restricted tissue distribution during the development. For example, the $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, and $\alpha 5(\text{IV})$ chains are present in the glomerular basement membrane (GBM) of the kidney, lung, testis, and eye, whereas the $\alpha 5(\text{IV})$ and $\alpha 6(\text{IV})$ chains are found in the BM of skin, smooth muscle, and the kidney. The expression of collagen IV chains is also subjected to temporal regulation. For instance, in the GBM of human kidney, genes encoding the $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ chains are expressed during early embryonic development (day 75), but their levels gradually decrease as the expression of genes encoding the $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, and $\alpha 5(\text{IV})$ chains starts. This developmental switch in gene expression is absolutely critical for the maturation of the GBM as a specialized plasma filtration barrier in the kidney. While most identified mutations in the genes encoding either $\alpha 1(\text{IV})$ or $\alpha 2(\text{IV})$ are embryonic lethal, most mutations in genes encoding the $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, or $\alpha 5(\text{IV})$ chains leads to renal failure and deafness in adult patients with Alport's syndrome (Hudson, 2004; Hudson et al., 2003).

Gene Organization and Regulation

Human collagen IV genes are arranged in three pairs with head-to-head orientation; *COL4A1-COL4A2* on chromosome 13, *COL4A3-COL4A4* on chromosome 2, and on chromosome X (Fig. 1). Sequence analyses from several mammalian species have pointed to a common ancestral gene, which through three consecutive gene duplications has resulted into six evolutionary related genes (Zhou et al., 1994). Based on sequence similarity, the genes can be divided into $\alpha 1$ -like group including *COL4A1*, *COL4A3*, and *COL4A5* genes, and $\alpha 2$ -like group comprising *COL4A2*, *COL4A4*, and *COL4A6* genes. The members of each group also share conserved sequence features such as characteristic exon–intron organization, indicating their evolutionary relationships. The bidirectional promoters shared by the paired genes is another unique feature of collagen IV, which is not shared by other collagen families. For example on the chromosome 13, *COL4A1* and *COL4A2* genes are transcribed from opposite DNA strands with transcription starting sites separated only by ~ 130 bp (Heikkila and Soininen, 1996). Similarly, the genes encoding $\alpha 5(\text{IV})$ and $\alpha 6(\text{IV})$ chains on chromosome X also share their promoter, however, the $\alpha 6(\text{IV})$ chain was shown to be encoded by two transcripts differing in their 5'-end sequences encoding different signal peptide (Oohashi et al., 1994; Sugimoto et al., 1994; Zhou et al., 1993). With no evidence for alternative splicing, these two transcripts were suggested to be regulated by two promoters (Sugimoto et al., 1994). Unlike other paired collagen IV genes, differential expression of *COL4A5* and *COL4A6* genes has been reported, indicating that transcription through alternative promoters may be used to differentiate *COL4A6* gene expression from that of *COL4A5* (Sugimoto et al., 1994).

Despite extensive studies and identification of the regulatory elements in the promoter regions, the regulation of collagen IV genes are not well understood. For example, the mRNA levels for $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ chains varies in different cells and under different conditions, yet the ratio of translated α -chains in the triple-helical $\alpha 1\alpha 1\alpha 2$ molecule remains always the same. This indicate that the expression of collagen IV genes are highly regulated on different levels such as transcription, stability and processing of encoding RNAs, efficiency of translation and posttranslational modifications, and last but not least, the chain-specific recognition of different α -chains in the cell. Alternative splicing is another

contributing factor to the complexity of chain composition and assembly of collagen IV α -chains. Analyses of $\alpha 3(\text{IV})$ chain in human kidney samples have revealed the presence of alternatively spliced transcripts, while no alternative spliced variants have been detected for the $\alpha 1(\text{IV})$, $\alpha 2(\text{IV})$, $\alpha 4(\text{IV})$, or $\alpha 6(\text{IV})$ chains (Bernal et al., 1993; Feng et al., 1994).

Structural Properties and Supramolecular Organization

In mammals, the collagen IV family comprises six highly homologous, yet genetically distinct α -chains (Fig. 1 and Table 1). Each chain contains three structurally distinct domains; an amino-terminal domain rich in cysteine and lysine residues, a major collagenous Gly-Xaa-Yaa triple repeats of $\sim 1,400$ residues, followed by a ~ 230 -residue long carboxy-terminal noncollagenous (NC1) domain (Hudson et al., 2003). The presence of cysteine- and lysine-rich residues at the amino terminus is essential for interchain crosslinking of four triple-helical molecules through disulfide bonds and lysine-hydroxylysine crosslinks. The crosslinked tetramer is heavily glycosylated making it resistant to collagenase activity. Following bacterial collagenase treatment of the BMs, the crosslinked tetramer can be isolated as a large molecular complex with a sedimentation coefficient of 7S, thus called the 7S domain (Risteli et al., 1980; Timpl et al., 1979). Another characteristic feature of collagen IV is the presence of 21–26 interruptions in the collagenous Gly-Xaa-Yaa triple repeats. The interruptions in the collagenous domain not only provide molecular flexibility for network formation but some of them also serve as cell-binding sites and interchain crosslinking (Vandenberg et al., 1991). The number of interruptions varies between chains, being the lowest in the $\alpha 1$ chain with 21 interruptions and the highest in $\alpha 4$ chain with 26 interruptions (Table 1).

The carboxy-terminal NC1 domain is also resistant to bacterial collagenase treatment and is easily isolated from collagenase treated BMs. The NC1 domains are the sites for molecular recognition through which the stoichiometry of chains in the assembly of triple-helical formation is directed (Khoshnoodi et al., 2006a). Study of the NC1 interactions have provided insights into chain-specific assembly of collagen IV chains, and therefore have been the focus of extensive study for the past three decades. Following treatment with bacterial collagenase, the NC1 domains can be purified from the BMs and studied as a hexamer. Recently, solution to crystal structure of the $[(\alpha 1)_2\alpha 2]_2$ NC1 hexamer has provided detailed structural information about the complex intermolecular interactions of the NC1 domains (Sundaramoorthy et al., 2002; Than et al., 2002). It was shown that each NC1 hexamer consists of two identical heterotrimers with a molecular stoichiometry of two $\alpha 1$ and one $\alpha 2$ NC1 domains. Both heterotrimers form a flat surface through which they interact end-to-end forming a compact ellipsoid-shaped molecule (Fig. 2). Since there are 12 conserved cysteine residues in each NC1 domain, it has long been thought that interchain disulfide bonds occur between different NC1 domains. However, it was revealed that the hexamer is stabilized through extensive hydrophobic and hydrophilic interactions without involvement of any di-sulfide bond. Furthermore, it was shown that the NC1 domains within each trimer interact through a three-dimensional domain swapping mechanism. In this type of interaction, the domain swapping motif (a β -hairpin structure) from each NC1 domain is “swapped” into a docking site (a four-stranded antiparallel β -sheet) located on the neighboring NC1 domain. Within each trimer, every NC1 domain “donates” a domain

swapping β -hairpin motif to one neighboring NC1 domain and accept a similar motif from another NC1 domain (Fig. 2). Such high degree of intermolecular complexity provides a high degree of specificity for chain selection through NC1 trimers, which function as a “folding origin” for triple-helical assembly of the entire collagen molecule. Thus the carboxy-terminal NC1 domains can be considered as the initial directors for the molecular assembly of the collagen IV molecules (Khoshnoodi et al., 2006a).

Once secreted into the extracellular matrix (ECM), the triple-helical molecules self-associate to form distinct networks providing a molecular scaffold onto which other ECM components such as laminins, perlecan, and proteoglycans can interact. The supramolecular structure of collagen IV network in situ was studied by Yurchenco and Ruben (1987) using high-resolution electron microscopy. Detailed analyses of high-resolution electron micrographs revealed that collagen molecules assemble to form irregular polygonal networks held together predominantly by overlapping lateral interactions along the triple-helical domain as well as the N-terminal and C-terminal end-domains (Fig. 2). More importantly, they showed that supercoil formation by supramolecular twisting is a characteristic feature of lateral associations which provides further stability to the network (Yurchenco and Ruben, 1987). Thus, through complex intermolecular interactions, these highly specialized ECM components form a remarkable set of tissue-specific BMs involved in many biological processes such as cell adhesion, migration and development, tissue regeneration and wound healing, reservoir of growth factors and enzymes, and in molecular sieving.

Biosynthesis and Modifications

Similar to other collagens, the biosynthesis of collagen IV is a complex process involving many co- and posttranslational modifications catalyzed by several specific and nonspecific enzymes (Myllyharju and Kivirikko, 2004). These enzymatic modifications are highly coordinated and include removal of the signal peptide in the endoplasmic reticulum (ER), hydroxylation of many proline and lysine residues, glycosylation of specific hydroxylysine residues, addition of mannose-rich oligosaccharide, and finally the formation of important intrachain disulfide bonds between the 12 conservative cysteine residues in the NC1 domain. In addition, biosynthesis of collagens is highly dependent on at least one specific molecular chaperon, Hsp47, without which proper folding will not take place (Matsuoka et al., 2004; Nagai et al., 2000).

In collagen IV, *N*-linked oligosaccharides are exclusively located in the 7S domain, whereas hydroxylysine-linked disaccharides are scattered along the entire molecule. The evolutionary conservation of *N*-linked glycosylation sites on the 7S domain in all chains and across different species strongly suggests that this posttranslational modification plays important role in the alignment and assembly of collagen IV network in the BMs (Langeveld et al., 1991; Nayak and Spiro, 1991). After alignments, the 7S domains are cross-linked through interchain disulfide bonds and perhaps also through covalent lysyl-hydroxylysine bonds, which introduce further stability to the collagen network.

Covalent crosslinks have also been reported for the C-terminal NC1 domains in the collagen IV networks. It has long been known that isolated NC1 hexamers can dissociate into

monomers and dimers by acid or other denaturant treatments. The presence of both reducible and nonreducible NC1 dimers have been repeatedly reported in different tissues such as kidney, placenta, aorta, and mouse Engelbreth-Holm-Swarm tumor (Boutaud et al., 2000; Langeveld et al., 1988; Siebold et al., 1988; Weber et al., 1984). Such dimers have long been assumed to be disulfide crosslinked monomers formed through a reshuffling mechanism of disulfide bonds during network formation (Netzer et al., 1998b; Siebold et al., 1988). However, the relative amount of nonreducible dimers isolated from different tissues varies significantly, for example 70% in BM of placenta but only 30% in BM of lens capsule (Langeveld et al., 1988; Vanacore et al., 2004).

The true nature of the crosslinked NC1 dimers and their tissue-dependent variation in relative amount has been puzzling for over two decades. Recent determination of X-ray crystal structure of NC1 hexamer reported from two independent laboratories have clearly disproved the interchain disulfide crosslink hypothesis (Sundaramoorthy et al., 2002; Than et al., 2002). Based on electron density maps at 1.9-Å resolution, however, a novel thioether crosslink between a methionine residue on one chain and a lysine residue on the opposite chain was proposed (Than et al., 2002). The chemical nature of this unusual covalent bond was further investigated by others. Based on trypsin digestion of separated monomer and dimer fractions, a crosslinked peptide unique to the dimer fraction was isolated and shown to derive from two $\alpha 1$ NC1 domains (Vanacore et al., 2005). Mass spectrometry and chemical analysis of the crosslinked peptide suggested that the crosslink occurred between the side chain of a methionine residue on one chain and a hydroxylysine on the other. The novel crosslink was named *S*-hydroxylysyl-methionine and suggested to be synthesized through two posttranslational modifications; hydroxylation of the lysine residue to hydroxylysine and formation of the covalent bonds between the hydroxylysine and sulfur atom of the methionine side chain. Disregard of the chemical nature and biosynthesis of this unusual crosslink, the presence of such unique covalent modification is most likely to be catalyzed by specific enzyme(s) present in a tissue-specific manner. Isolation and characterization of such posttranslation modifying enzyme(s) will be another intriguing research area in the biosynthesis of collagens.

Discovery of Chains and the Question of Assembly

A common feature among collagens of all types is that, they consist of three polypeptide chains (α -chains) with repeating Gly-X-Y sequences which have high propensity to form supercoiled triple-helical structure (Brodsky and Ramshaw, 1997; Brodsky and Shah, 1995; Jenkins and Raines, 2002). Some collagens, such as collagen III, are obligate homotrimers comprising three identical α -chains, whereas others form heterotrimers containing at least two different α -chains (Prockop and Kivirikko, 1995). As ubiquitous as they are, many types of collagens are expressed in the same cell, yet in the ER they are all assembled in a type- and chain-specific manner with correct stoichiometry before they are secreted into the ECM.

Similar to many collagens, the assembly of collagen IV starts from the carboxy-terminal ends, where specific interactions between NC1 domains direct the stoichiometry of chains in a triple-helical molecule. While assembly of other heterotrimeric collagen types involves

recognition between two or three different α -chains, assembly of the collagen IV molecules includes selection among six different α -chains. Theoretically, these six α -chains could assemble into 76 different combinations including six homotrimers and 70 heterotrimers. However, only three specific heterotrimers ($\alpha 1\alpha 1\alpha 2$, $\alpha 3\alpha 4\alpha 5$, and $\alpha 5\alpha 5\alpha 6$) have so far been found in all tissues studied (Borza et al., 2001; Boutaud et al., 2000; Hudson et al., 1994, 2003). This finding reflects a remarkable specificity among the α -chains of collagen IV, and raises a fundamental question about the underlying mechanism in the chain selection and formation of different heterotrimers. The extraordinary ability for chain selection makes the collagen IV the “prototype” molecule to delineate the mechanism of chain selection in the assembly of collagens. In addition, the detailed structural information which was recently obtained through solution of its NC1 crystal structure makes collagen IV an ideal molecule to decipher the structural basis of the sites involved in chain recognition and selection. The assembly question has been subjected for intensive investigation through the years. Here we describe some of the highlights.

Evolution of Assembly Question

In 1981, the first network model for the organization of collagen IV was reported (Timpl et al., 1981). Using rotary shadowing technique, it was demonstrated that individual collagen IV molecules appeared as 390-nm long strands with a globular domain which was termed noncollagenous domain (NC1). Two of such molecules could form a dimer through interaction of their NC1 domain. In protein fractions that had been solubilized by pepsin digestion, the NC1 domains disappeared and the remaining molecules mainly consisted of four triple-helical strands joined together through the amino terminal 7S domains. Thus it was demonstrated that collagenous network of BMs consists of collagen IV molecules with two different interacting sites; a 7S domain which connects four molecules and the NC1 domain which connects two molecules. Soon after, it was shown that the NC1 domain consisted of $\sim 30,000$ molecular weight polypeptide located at the carboxyl end of the molecule and contained intramolecular disulfide bridges (Fessler and Fessler, 1982).

Until mid 80s, only two α -chains of collagen IV were known to exist. However, based on chemical analysis of the GBM, the existence of minor but different collagen IV subunits was known for several years. Following digestion of the GBM with bacterial collagenase and chemical analysis of isolated NC1 domains, evidence for the existence of two novel α -chains, named $\alpha 3(\text{IV})$ and $\alpha 4(\text{IV})$, with different amino acid sequences than those derived from the classical $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ chains were reported (Butkowski et al., 1985, 1987; Wieslander et al., 1985). Based on partial peptide sequences derived from isolated NC1 domains of human GBM (Butkowski et al., 1990; Gunwar et al., 1990; Kleppel et al., 1986; Saus et al., 1988), partial cDNA clones were soon isolated by PCR amplification (Mariyama et al., 1992; Morrison et al., 1991a,b). Shortly, this was followed by characterization of the complete primary structure of the human $\alpha 3(\text{IV})$ and $\alpha 4(\text{IV})$ chains and their genes were localized to human chromosome 2 (Leinonen et al., 1994; Mariyama et al., 1994; Quinones et al., 1992; Turner et al., 1992). With the discovery of $\alpha 5(\text{IV})$ and $\alpha 6(\text{IV})$ chains by molecular cloning, the fundamental question of chain-specificity was revived. The ability of collagen IV chains to self-assemble through interactions at their NC1 domains had already been reported in early 80s (Timpl et al., 1981). Pepsin-treated molecules which lacked their

NC1 domains were also shown to lack the ability to form network (Yurchenco and Furthmayr, 1984). Using rotary shadowing technique, a time-dependent reassembly of heat-denatured collagen IV molecules was clearly visualized, demonstrating a zipper-like refolding fashion of the triple-helical molecule starting from the C-terminal NC1 domains and proceeding toward the N-terminal ends (Dolz et al., 1988). Direct evidence that the chain specificity of collagen assembly is encoded by the NC1 domains was first demonstrated using purified NC1 hexamers isolated from BMs of different tissues (Boutaud et al., 2000). It was shown that acid-dissociated NC1 hexamers had the ability to reassemble back to native configuration with the original molecular stoichiometry. More interestingly, it was shown that hexamer formation from the $\alpha 3$, $\alpha 4$, and $\alpha 5$ NC1 monomers required the presence of all three NC1 domains, further suggesting that mutations in individual NC1 domains may interrupt the assembly of the $\alpha 3\alpha 4\alpha 5$ network. All these findings clearly highlighted the critical role of the NC1 domain in the assembly of collagen IV molecules, and suggested that the NC1 domains contain recognition sequences for chain selection.

Using detailed kinetics and crystal structure analysis, we have recently reported the molecular mechanism of chain-selection in the assembly of collagen IV (Khoshnoodi et al., 2006b). Using a surface plasmon resonance biosensor, the kinetic of interaction and assembly of isolated $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$ NC1 domains were determined. The analysis showed that the $\alpha 2(\text{IV})$ NC1 domain had a prominent role in regulating the stoichiometry of chains and assembly of $\alpha 1\alpha 1\alpha 2$ hetero-trimer, by displaying a significantly higher affinity for heterodimer formation with $\alpha 1(\text{IV})$ NC1 domain than with itself. As the chain-specific assembly of the collagen IV molecules is initiated by the early interactions of NC1 domains in the ER, this characteristic differential affinity of certain NC1 domains for dimer formation may play a major regulatory role in determining the stoichiometry of chains in a particular collagen IV molecule. Moreover, based on data derived from the atomic structure of the NC1 hexamer, two prominent regions which together provide specificity for chain selection were identified. These regions comprised a 13-residue long β -hairpin motif involved in the domain-swapping mechanism stabilizing the intermolecular interaction between two NC1 domains, and a 15-residue long region with genetic hypervariability (VR3) which mediates chain-specific recognition between two NC1 domains (Fig. 2) (Khoshnoodi et al., 2006b). Thus it is most likely that chain-specific assembly of triple-helical collagen IV molecules in the ER is governed mainly by two factors: (i) specific recognition sites located on each NC1 domain and (ii) differential affinity of particular α -chains for dimer formation. Based on sequence identity, both $\alpha 4(\text{IV})$ and $\alpha 6(\text{IV})$ chains belong to the $\alpha 2$ -like subtype and also occur only once in $\alpha 3\alpha 4\alpha 5$ and $\alpha 5\alpha 5\alpha 6$ trimers, respectively. Therefore, it is likely that these chains also play a regulatory role in the assembly of their corresponding triple-helical molecules.

Interaction To Cell Surface Receptors

In addition to providing a scaffold for assembly and mechanical stability, collagen IV is an important component in interaction of cells with underlying basement membrane. This interaction is critical for a variety of biological processes, including cell adhesion, migration, survival, proliferation, and differentiation. Cell culture studies have shown that collagen IV is the binding substrate for a large number of cell types including platelets (Santoro, 1986;

Staatz et al., 1990), hepatocytes (Rubin et al., 1981), keratinocytes (Murray et al., 1979), endothelial (Cheng and Kramer, 1989; Herbst et al., 1988), mesangial (Setty et al., 1998), pancreatic (Kaido et al., 2004), and tumor cells such as breast and prostate carcinoma (Abecassis et al., 1987; Dedhar et al., 1993), melanoma (Chelberg et al., 1989), fibrosarcoma, and glioma (Aumailley and Timpl, 1986; Knight et al., 2000). Cell attachment to collagen IV is mediated by multiple binding sites within both triple-helical and NC1 domains, suggesting involvement of several adhesion receptors (Chelberg et al., 1989; Herbst et al., 1988; Wayner and Carter, 1987). These receptors can be divided into integrin and nonintegrin receptors.

Integrin Receptors

Integrins comprise a large number of structurally and functionally related transmembrane glycoproteins which are the major cell surface receptors for the ECM (Hynes, 1987, 1992, 2002). On the cell surface, they are present as noncovalent heterodimers consisting of an α - and a β -subunit. Each subunit has a large extracellular domain, a short transmembrane segment, and a C-terminal cytoplasmic tail with affinity for cytoskeletal proteins. Thus the term integrins reflect their ability to “integrate” cells into their immediate environment by connecting the cytoskeleton with the extracellular components. So far, 18 α - and 8 β -subunits have been identified in mammalian cells and are known to interact in a tissue-restricted manner to form 24 distinct family members (Humphries et al., 2006; Hynes, 2002). This diversity is further expanded by alternative splicing, posttranslational modifications, and interaction to other cell surface receptors and intracellular molecules (de Melker and Sonnenberg, 1999; Green et al., 1998; Porter and Hogg, 1998).

An important characteristic feature of integrins is their ability to recognize short peptide sequences within large ECM proteins. The classical example of integrin binding site is the Arg-Gly-Asp (RGD) sequence originally identified in fibronectin, which is a common ligand for at least eight different integrins (Ruoslahti, 1996). Although multiple RGD sequences exist within the triple-helical region of several collagen IV α -chains, several studies have shown that cell adhesion to collagen IV is RGD-independent (Herbst et al., 1988; Kim et al., 1994; Kramer and Marks, 1989). A possible explanation is that due to their triple-helical nature, these RGD sequences are not accessible to integrins. However, cell adhesion to collagen IV is predominantly dependent on triple-helical state of collagen IV, since reduction of disulfide bonds followed by heat-denaturation dramatically decreases cell binding and spreading (Aumailley and Timpl, 1986; Perris et al., 1993; Santoro, 1986).

The major collagen receptor include the β_1 subgroup of integrins, namely $\alpha_1\beta_1$ and $\alpha_2\beta_1$ (Aumailley and Gayraud, 1998; Leitinger and Hohenester, 2007; Paulsson, 1992). These two integrins bind both collagen IV and collagen I, however, with distinct specificity as follows: integrin $\alpha_1\beta_1$ has a higher affinity for collagen IV, while $\alpha_2\beta_1$ binds stronger to collagen I (Kern et al., 1993; Tulla et al., 2001a). It was shown that deletion of $\alpha_1\beta_1$ integrin by the homologous recombination resulted in dramatic decrease in adhesion and migration of fibroblasts and smooth muscle cells to collagen IV substrate (Gardner et al., 1996), while the importance of $\alpha_2\beta_1$ integrin was demonstrated by the decrease of adhesion and morphogenesis on collagen IV after using antisense mRNA (Keely et al., 1995). A major

binding site for $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins has been originally identified within a triple-helical cyanogen bromide-derived fragment CB3, located 100 nm away from the amino-terminus of the collagen IV (Fig. 3) (Vandenberg et al., 1991). The triple-helical conformation of this particular region of the molecule is stabilized through interchain disulfide bridges between the $\alpha_1(\text{IV})$ and $\alpha_2(\text{IV})$ chains. Using antibodies to CB3 fragment, it was shown that about 80% of cell adhesion to collagen IV was inhibited, suggesting that it comprises a major cell binding region. By affinity chromatography using immobilized CB3 fragments and cellular lysates, $\alpha_1\beta_1$ and $\alpha_2\beta_1$ were isolated as the only integrins eluted from the affinity column (Vandenberg et al., 1991). Using purified integrins and shorter tryptic fragments of CB3, one binding site for $\alpha_1\beta_1$ integrin and two for $\alpha_2\beta_1$ integrin were located in different but adjacent positions on CB3 fragment (Kern et al., 1993). Further refinement of the $\alpha_1\beta_1$ recognition site was achieved by digestion of one of the tryptic peptides with thermolysin (Eble et al., 1993). Thus, the recognition site within CB3(IV) fragment was located within a conformational-dependent region, where the spatial vicinity of Asp461 on $\alpha_1(\text{IV})$ chain to Arg461 on $\alpha_2(\text{IV})$ chain showed to be critical for binding of $\alpha_1\beta_1$ integrin. Finally, functional activity of this site for $\alpha_1\beta_1$ integrin binding has been demonstrated directly using synthetic triple-helical peptide containing 12 amino acid residues (457–468) from both α_1 and α_2 chains of collagen IV stabilized with artificial cysteine knot (Renner et al., 2004).

Similar to $\alpha_1\beta_1$, a conformational-dependent binding to collagen I and IV has been shown for $\alpha_2\beta_1$ integrin (Knight et al., 1998). The $\alpha_2\beta_1$ integrin plays an important role in platelet adhesion to collagens and hemostasis in the blood vessel wall (Sixma et al., 1997). It has been shown that a short sequence of GFOGER peptide (O being hydroxyproline) represents a binding site for the $\alpha_2\beta_1$ integrin (Knight et al., 2000). The sequence represents a high-affinity binding site in both collagen I and IV, and is entirely dependent on the native triple-helical conformation. Replacement of phenylalanyl or glutamyl residues abolished the binding, indicating their important role for recognition. Triple-helical GFOGER peptide is a potent inhibitor of cell adhesion to collagen IV. Moreover, this sequence is present in α_1 chain of CB3(IV) fragment (385–390), where it might represent one of the two binding sites for $\alpha_2\beta_1$ integrin (Fig. 3).

In addition to $\alpha_1\beta_1$ and $\alpha_2\beta_1$, other integrins may also participate in cell binding to collagen IV. Cell adhesion to collagen IV mediated by $\alpha_3\beta_1$ integrin was shown for small lung carcinoma cell line expressing low levels of other β_1 integrins (Elices et al., 1991). In addition, α_3 subunit antisense oligonucleotide partially inhibited adhesion of prostate carcinoma cells to collagen IV (Leung-Hagesteijn et al., 1994). One binding site for $\alpha_3\beta_1$ integrin has been identified on α_1 chain of collagen IV (residues 531–543) using synthetic peptide GEFYFDLRLKGDK, which promoted adhesion of several tumor cell lines and induced $\alpha_3\beta_1$ -mediated signal transduction (Lauer et al., 1998; Miles et al., 1994, 1995). However, the role of $\alpha_3\beta_1$ as a collagen IV receptor remains controversial since integrin-specific antibody failed to block the adhesion of various cells (Elices et al., 1991; Melchiori et al., 1995), and overexpression of $\alpha_3\beta_1$ does not increase adhesion to the collagen IV (Delwel et al., 1994; Weitzman et al., 1993). Most surprisingly, genetic ablation of α_3 integrin increases adhesion and migration of keratinocytes on collagen IV leading to the hypothesis that $\alpha_3\beta_1$ is a trans-dominant inhibitor of collagen IV binding integrins (Hodivala-Dilke et al., 1998). Finally, the $\alpha_3\beta_1$ integrin complex, either as recombinant or

purified from cells, has failed to bind collagen IV in vitro (Eble et al., 1998; Pochec et al., 2003).

Recently, binding of two new integrins, $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$, to collagen IV have been reported (Tiger et al., 2001; Tulla et al., 2001b). The α_{11} I domain binds stronger to collagen I through the same GFOGER motif as $\alpha_2\beta_1$ integrin (Zhang et al., 2003), while binding of α_{10} is more specific for collagen IV similar to α_1 I domain (Tulla et al., 2001b). Nevertheless, structure of binding sites for these two integrins on collagen IV remains unknown. Expression of $\alpha_{10}\beta_1$ and $\alpha_{11}\beta_1$ is spatially and temporally restricted to chondrocytes and fetal muscle cells, suggesting their specific role in development.

Thermal denaturation or proteolytic degradation results in exposure of novel binding sites on collagen molecules. This phenomenon was first described for collagen I, which after thermal denaturation binds fibronectin with increased affinity (Engvall and Ruoslahti, 1977; Schor and Court, 1979), and later for collagen IV (Aumailley and Timpl, 1986). These results led to suggestion that cell adhesion to denatured collagen could be mediated by fibronectin- $\alpha_5\beta_1$ integrin bridge (Tuckwell et al., 1994). More recent studies show the exposure of a cryptic site within the triple-helical part of the collagen IV after proteolytic cleavage (Xu et al., 2001). This cleavage occurred in blood vessels during angiogenesis and was associated with a loss of $\alpha_1\beta_1$ and concomitant gain of $\alpha_v\beta_3$ integrin binding. Moreover, monoclonal antibodies to denatured collagen IV inhibited angiogenesis and tumor growth in vivo. These results suggest that degradation and proteolytic remodeling of BMs might expose cryptic sites within collagen IV with altered integrin specificity and biological functions.

In addition to the triple-helical part, NC1 domain of collagen IV also promotes cell adhesion (Chelberg et al., 1989; Herbst et al., 1988; Setty et al., 1998), stimulates outgrowth of embryonic neurons (Lein et al., 1991), and inhibits morphogenesis in *Hydra vulgaris* (Zhang et al., 1994). The latter finding led to the suggestion that NC1 domain could inhibit angiogenesis by disrupting the assembly of vascular basement membrane (Sarras and Hudson, 1997). Subsequent studies with purified recombinant NC1 domains demonstrated that α_2 , α_3 , and α_6 NC1 domains promote the adhesion and migration of endothelial cells, while α_1 , α_4 , and α_5 NC1 domains were inactive (Petitclerc et al., 2000). Interestingly, systemic administration of α_1 , α_2 , and α_3 NC1 domains also inhibits tumor growth and angiogenesis in vivo (Colorado et al., 2000; Maeshima et al., 2000b; Petitclerc et al., 2000). Inhibition of cell adhesion with neutralizing antibodies suggests that different NC1 domains may bind distinct integrins: $\alpha_1\beta_1$ for α_1 NC1, $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_3\beta_1$ for α_2 NC1, and $\alpha_v\beta_3$ for α_3 and α_6 NC1 domains (Fig. 3). Somewhat different results were obtained for melanoma and ovarian carcinoma cells which interact with α_2 NC1 domain through $\alpha_1\beta_1$ and $\alpha_v\beta_3$ integrins (Roth et al., 2005). Recently, we directly identified $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins as endothelial receptors for α_3 NC1 domain by affinity chromatography and confirmed binding of purified integrins in vitro (Pedchenko et al., 2004). So far, specific integrin binding sites have been characterized only for α_3 NC1 domain (Han et al., 1997; Maeshima et al., 2000a). One region was initially identified within the first half of α_3 NC1 domain (residues 54–132), since corresponding recombinant protein inhibited proliferation of endothelial cells, induced apoptosis, and decreased tumor growth in nude mice (Maeshima et al., 2001a). Subsequently, shorter synthetic peptide derived from this region (residues 74–98) was

shown to inhibit proliferation of endothelial cells presumably via interaction with $\alpha_v\beta_3$ integrin in a RGD-independent manner (Maeshima et al., 2001b). This results in inhibition of the Cap-dependent protein synthesis mediated by focal adhesion/phosphatidylinositol-3/Akt/mTOR kinases (Maeshima et al., 2002; Sudhakar et al., 2003). A second cell-binding site was ascribed to amino acids 185–203 of α_3 NC1 domain based on the inhibition of melanoma cells proliferation by the corresponding synthetic peptide (Han et al., 1997). This peptide binds $\alpha_v\beta_3$ integrin and CD47/IAP from the cell lysate through specific interaction of β_3 subunit with amino acid triplet SNS (Pasco et al., 2000; Shahan et al., 1999). In addition, we recently found that endothelial cell adhesion to recombinant α_3 NC1 is critically dependent on the interaction of the adjacent amino terminal RGD motif with $\alpha_v\beta_3$ integrin as deletion of RGD sequence significantly decreased cell adhesion, and the two above-mentioned peptides were unable to recapitulate cell adhesion to α_3 NC1 domain (Pedchenko et al., 2004). Thus, NC1 domains of collagen IV act as ligands for distinct integrins through several binding sites that may mediate their anti-angiogenic and anti-tumor activities.

Interaction of cells with 7S domain of collagen IV has been reported by several groups, suggesting the existence of an additional yet uncharacterized binding site for cell adhesion receptors. Purified 7S domain efficiently blocks cell aggregate development in Hydra, suggesting that it may play an important role in morphogenesis (Zhang et al., 1994). In another report, the 7S domain potently stimulated chemotaxis in neutrophils, and this response is potentially mediated by a 67-kD lectin-like protein similar to the elastin receptor (Senior et al., 1989).

Nonintegrin Receptors

Evidence has also been reported for cell-collagen IV interaction through nonintegrin receptors, such as cell-surface heparin sulfate proteoglycans, glycoprotein VI and mannose receptor family (Leitinger and Hohenester, 2007). Melanoma cells expressing the chondroitin sulfate proteoglycan CD44 bind directly to collagen IV, and cell interaction and migration could be inhibited by anti-CD44 antibodies (Knutson et al., 1996). Using synthetic triple-helical peptides, the binding site for CD44 has been localized to a 14-residue long region within the $\alpha_1(\text{IV})$ chain (Lauer-Fields et al., 2003). In addition, three heparin-binding sequences have also been found in the triple-helical and NC1 domains of $\alpha_1(\text{IV})$ and $\alpha_2(\text{IV})$ chains (Koliakos et al., 1989). A synthetic peptide (TAGSCLRKFSTM) corresponding to a sequence from $\alpha_1(\text{IV})$ NC1 domain has been shown to promote adhesion and spreading of endothelial cells (Tsilibary et al., 1990). Another heparin-binding peptide, Hep-III (GEFYFDLRLKGDK), derived from the interruption of α_1 chain promoted adhesion of keratinocytes and mesangial cells (Setty et al., 1998; Wilke and Furcht, 1990). Interestingly, the same peptide mediates binding of $\alpha_3\beta_1$ integrin and may also interact with $\alpha_2\beta_1$ integrin depending on integrin expression levels (Kim et al., 1994; Setty et al., 1998). However, the degree of cell spreading on Hep-III is significantly lower than on full-length type IV collagen or its triple-helical domain, and Hep-III only partially inhibited cell motility on collagen IV, indicating rather limited contribution of proteoglycans to mechanisms of cell adhesion and migration.

Another group of collagen-binding proteins called discoidin domain receptors (DDR) has recently been discovered (Shrivastava et al., 1997; Vogel et al., 1997). DDRs belong to the large family of receptor tyrosine kinases, and two members of this family, DDR1 and DDR2, are widely expressed in mammalian cells. Their most distinctive feature is the stimulation of tyrosine kinase activity by native triple-helical collagens that sustain over an extended period of time. Interestingly, collagen IV stimulates phosphorylation of DDR1, but not DDR2. Activation of DDR1 was not affected in the β_1 integrin null cells, suggesting that DDR1 represents independent receptor directly interacting with collagen (Vogel et al., 2000). This does not exclude a possibility of further downstream crosstalk between integrin and DDR signaling pathways. Deletion of DDR1 led to decreased collagen adhesion, migration of vascular smooth muscle cells, and enhanced proliferation of mesangial cells (Curat and Vogel, 2002; Hou et al., 2001). In addition, DDR1 knockout mice develop localized thickening of GBM and proteinuria, suggesting that collagen IV-DDR1 interaction plays an important role for structural integrity and filtration function in the kidney (Gross et al., 2004).

Genetic and Acquired Diseases of Collagen IV

Collagen IV is involved in a number of genetic and acquired disorders. Depending on genetic and nongenetic factors including alterations in temporal and spatial gene expression, splice variations, posttranslational modifications, and the chain-specific assembly of particular α -chains, different organs can be affected during the development and in the adult life span. Among these organs, the kidney has shown to be particularly affected by collagen IV abnormalities. Because of its highly specialized features, the BM of the renal glomerulus plays a critical role in filtration of the circulating plasma. As all the six collagen IV genes are differentially expressed in different anatomical parts of the glomerulus, and since lack of one gene product can not be compensated by another, renal disorders of collagen IV are the most life-threatening disorders leading to end-stage-renal-disease (ESRD) and mortality. Alport's and Goodpasture's syndromes are the two major examples of the kidney abnormalities in which collagen IV is the central molecule directly involved in the pathogenesis, and thus will be discussed here.

Alport's Syndrome

In 1927, Arthur Cecil Alport described the clinical syndrome of a hereditary nephritis characterized by hematuria and sensorineural deafness (Alport, 1927). For decades, the syndrome was diagnosed solely based on its clinical manifestations. However, with advances in electron microscopy in the early 1970s, the underlying pathological clues to the renal dysfunction, which was ultrastructural changes with regional thinning and thickening of the GBM, were reported from several laboratories (Churg and Sherman, 1973; Hinglais et al., 1972; Kinoshita et al., 1969; Spear and Slusser, 1972). The presence of a unique collagen in the GBM had already been reported by Kefalides in the late 60s (Kefalides, 1966, 1968), and it was thought to consist of three identical α -chains forming the scaffold of the GBM (Kefalides, 1971). This inspired Spear to suggest that mutations in the gene for this unique structural GBM component could cause Alport's syndrome (Spear, 1973). However, it was not until 1990 that the genetic basis for what came to be known as X-linked

Alport's syndrome was described (Barker et al., 1990; Hostikka et al., 1990; Myers et al., 1990; Pihlajaniemi et al., 1990). Since then, a large number of different mutations in the COL4A5 gene as well as in the COL4A3 and COL4A4 genes have been reported (Jais et al., 2000, 2003; Longo et al., 2002). These mutations not only include nonsense and missense mutations, but also mutations that affect splicing, stability of triple-helical structure, posttranslational modifications, and the assembly of chains into heterotrimeric molecules. While mutations in the COL4A3 or COL4A4 genes cause autosomal recessive form of the syndrome affecting both males and females equally (Lemmink et al., 1994; Mochizuki et al., 1994), ~85% of patients with Alport's syndrome carrying mutations in the COL4A5 gene and therefore are classified as X-linked with the highest prevalence in males (Barker et al., 1990; Martin et al., 1998).

The expression of genes encoding the $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, and $\alpha 5(\text{IV})$ are subjected to developmental switch in the GBM. Thus, disregard of which gene is affected mutations that alter normal expression, posttranslational modifications or assembly of the $\alpha 3\alpha 4\alpha 5$ triple-helical molecule will cause an arrest in the developmental switch and persistence of the $\alpha 1\alpha 1\alpha 2$ network in the GBM with Alport's syndrome as consequence. Differences in the physical and biological properties between the classical $\alpha 1\alpha 1\alpha 2$ network and the $\alpha 3\alpha 4\alpha 5$ network are most likely to underlie the disability of the former network to compensate for the lack of the latter in the Alport's syndrome patients. Relative to $\alpha 1\alpha 1\alpha 2$ network, the $\alpha 3\alpha 4\alpha 5$ network is highly crosslinked because of its larger degree of inter- and intrachain disulfide bonds and has different physical properties than the classical $\alpha 1\alpha 1\alpha 2$ network (Gunwar et al., 1998). During the embryonic development of the kidney and the increased plasma filtration cross the glomerular capillaries, the levels of circulating proteins including serum proteases constantly increases. The higher susceptibility of the embryonic $\alpha 1\alpha 1\alpha 2$ network to proteolytic activities makes the developmental switch to the more resistant $\alpha 3\alpha 4\alpha 5$ network necessary to withstand higher glomerular pressure and proteolytic degradation of the GBM. Patients with Alport's syndrome probably become more sensitive to gradual proteolytic degradation resulting in uneven thickness of the GBM characterized in all renal samples of Alport's syndrome patients.

Patients with Alport's syndrome can benefit from renal transplantation as the development of nephritis due to generation of alloantibodies is not very common. Sensitive assays have shown that the posttransplant alloantibodies are always directed against multiple epitopes along the $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, or $\alpha 5(\text{IV})$ chains (Hudson et al., 1992; Kalluri et al., 2000). Circulation of such antibodies is a characteristic feature of another collagen IV-related renal disease known as Goodpasture's syndrome.

Goodpasture's Syndrome

Goodpasture's syndrome is an autoimmune disease characterized by a rapidly progressive glomerulonephritis which is often associated with lung hemorrhage. The disease, which occurs primarily in young men usually in their late 20s and in both men and women in their 60s, is caused by circulating autoantibodies directed to specific epitopes located on the $\alpha 3(\text{IV})$ NC1 domain (Saus et al., 1988; Wieslander et al., 1984). Although immunoreactivity to epitopes located on the NC1 domains of other collagen IV chains have been reported,

almost all anti-GBM antibodies from patients are reactive to the $\alpha 3(\text{IV})$ NC1 domain (Kalluri et al., 1995). In this regard, the immunospecificity of antibodies to $\alpha 3(\text{IV})$ NC1 domain is puzzling, since antibodies to NC1 domains of other chains, such as $\alpha 1(\text{IV})$ and $\alpha 2(\text{IV})$, do not cause anti-GBM nephritis (Bolton et al., 1995; Kalluri et al., 1996; Sado et al., 1998). Genetic factors have been reported indicating that patients with certain allelic variations (HLA-DRB1*1501 and DRB1*1502) have increased susceptibility to develop the disease, whereas other alleles (HLA-DR7 and DR1) are protective (Phelps and Rees, 1999). The role of autoreactive CD4+ T cells in mediating the disease has been reported, clearly indicating the importance of cell-mediated autoimmunity in the pathogenesis (Salama et al., 2001; Wong et al., 2001).

The pathogenesis of Goodpasture's syndrome is characterized by glomerulonephritis which is initiated via high-affinity autoantibodies, usually of the IgG class. Two dominant epitopes (E_A and E_B) within the $\alpha 3(\text{IV})$ NC1 domain have been identified (Borza et al., 2000; David et al., 2001; Gunnarsson et al., 2000; Hellmark et al., 1999; Netzer et al., 1999). Located in close proximity to each other and near the triple-helical junction (Fig. 2), these epitopes do not normally show reactivity with the antibodies unless the NC1 hexamer is dissociated (Borza et al., 2002). Therefore, it is presumed that environmental factors such as exposure to hydrocarbons or tobacco smoke may be needed to reveal the cryptic epitopes to the immune system (Donaghy and Rees, 1983; Stevenson et al., 1995). Disregard of the cause, binding of immunoglobulins to the GBM activates the complement system and a cascade of proteases, which results in disruption of the glomerular filtration barrier followed by proteinuria and formation of glomerular crescent. The cell-mediated immunoreactivity by CD4+ and CD8+ T cells is soon followed by migration of macrophages and neutrophils into the glomerulus causing further inflammation and increased proteinuria which lead to interstitial nephritis (Kitching et al., 2002; Remuzzi and Bertani, 1998; Timoshanko et al., 2001; Wu et al., 2001, 2002).

Treatments for Goodpasture's patients are limited to plasmapheresis which removes the harmful antibodies from the circulations and/or immunosuppression. Kidney transplantation is possible, however, because there is a risk for recurrence the procedure should wait until anti-GBM antibodies are undetectable in the serum (Netzer et al., 1998a).

Rare Genetic Disorders of Collagen IV

Recently, it was shown that mutations in the *COL4A1* gene causes type 1 or encephaloclastic porencephaly, which is a rare neurological disease typically manifested in infants (Gould et al., 2005). The disease is characterized by the presence of degenerative cavities and cerebral lesions in the brain leading to severe pathological disorders such as poor speech development, epilepsy, mental retardation, and cerebral palsy (Pasternak et al., 1980). Using a mouse model generated by random mutagenesis, Gould et al. characterized a novel mouse mutant with severe perinatal cerebral hemorrhage similar to those previously described in a large human family with autosomal dominant type 1 porencephaly (Aguglia et al., 2004; Smit et al., 1984). The causative gene in the mouse mutant was mapped and identified as *COL4A1* gene. Sequence analysis revealed that a splice site mutation which resulted in excision of an exon generated a semidominant mutant $\alpha 1(\text{IV})$ chain, which upon assembly

inhibited secretion of the wild-type and mutant collagen IV (Gould et al., 2005). Electron micrographs of the mutant mice clearly demonstrated structural defects in the cerebral vasculature such as uneven BMs with variable thickness, inconsistent density, focal disruptions, and herniation which presumably reflect a weaker BM structure. Because not all mutant mice developed porencephaly, the authors proposed that in addition to the mutations environmental factors such as birth trauma may be needed to cause the disease (Gould et al., 2005).

The above finding tempted the authors to investigate potential mutations in the human *COL4A1* gene in two ethnically separate families with autosomal dominant porencephaly. Sequence analysis revealed two mutations (Gly to Arg and Gly to Ser) leading to replacement of a conserved Gly residue in the Gly-Xaa-Yaa repeats of the triple-helical collagenous domain. Such mutations are known to disrupt the triple-helical formation during the assembly of collagens leading to protein defect (Engel and Prockop, 1991). Interestingly, in addition to porencephaly, one of the adult patients was found to suffer from recurrent hemorrhagic strokes caused by weakened vascular BM. This finding indicates that such dominant mutations in the *COL4A1* gene may also contribute to hemorrhagic stroke in adults (Gould et al., 2005). Indeed, such indications have been reported in an adult patient with autosomal dominant porencephaly who showed only minor neurological dysfunction, but suffered from recurrent strokes (van der Knaap et al., 2006). These findings suggest that certain mutations or polymorphisms in the *COL4A1*, *COL4A2*, or the genes encoding other BM components may conspire with environmental factors to underlie recurrent hemorrhagic strokes in adults.

Conclusion

Four decades after its discovery, collagen IV is still the focus of extensive research from biochemistry to pathology and genetic disorders. Being the only collagen type encoded by six different genes, the six α -chains of collagen IV can recognize each other with a remarkable specificity and assemble into unique heterotrimers. After secretion into the ECM, these molecules further interact to form higher supramolecular organizations which together with other proteins finally form unique BMs in a tissue-specific manner. Through interactions with specific cellular receptors such as integrins, the BM collagen IV networks not only provide structural support to the cells and tissues, but they also affect the biological fate during and after the development. As new discoveries keep unraveling mysteries about the genetic mutations, biosynthesis, molecular assembly, and network formation of collagen IV, our understanding has dramatically increased about the critical role of these complex supramolecular structures in health and disease.

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Fig. 1.

Schematic view of gene localization, organization, gene product, and assembly of six different isoforms of human collagen IV chains. The genes, *COL4A1/COL4A2* on chromosome 13 (**A**), *COL4A3/COL4A4* on chromosome 2 (**B**), and *COL4A5/COL4A6* on chromosome X (**C**), are organized in pair sitting head-to-head and separated by a short promoter region that both genes share. The paired genes are transcribed in a bidirectional fashion and translated into individual α -chains. Each chain starts with an N-terminal 7S domain having an N-linked oligosaccharide (Y-shaped and black), a long collagenous domain, and a C-terminal globular noncollagenous domain (NC1). Assembly of each heterotrimer with characteristic stoichiometry of chains initiates by chain-specific recognition of NC1 domains, formation of NC1-trimers followed by supercoiling of the triple-helical collagenous domains which proceeds toward the N-terminal 7S domains. Out of 56 potential heterotrimers, only three specific combinations: $\alpha1\alpha1\alpha2$, $\alpha3\alpha4\alpha5$, and $\alpha5\alpha5\alpha6$, have been found in vivo.

Fig. 2. Generic network formation and structural features of the NC1 domain. A heterotrimeric collagen IV molecule (**A**) can interact through its N-terminal 7S domains to form a tetramer (left) or through its NC1 domains to form a dimer (right). Through complex interactions, these molecules can further interact to form higher order of supramolecular organization and three-dimensional networks. The networks are further enforced by supramolecular twisting and lateral associations of collagenous domains (arrow heads). Domain organization and configuration of the NC1 domains in hexamer and trimer (**B**). The NC1 hexamer is formed by end-to-end interaction of two NC1 trimers. Relative location of two immunologically reactive epitopes, EA (yellow) and EB (orange) on the alpha3(IV) NC1 domain are shown. Each NC1 trimer is stabilized by a three-dimensional domain swapping mechanism through tight and selective interactions between two sites comprising a beta-hairpin motif and its docking site with a variable region (VR3).

Fig. 3.

Location of integrin-binding sites within three different collagen IV heterotrimers. Binding sites within the triple-helical cyanogen bromide-derived fragment (CB3) and specific NC1 domains are indicated.

Table 1
Structural features of the six α -chains of human collagen IV

	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$
Gene localization on chromosome	13	13	2	2	X	X
Residues after translation	1,669	1,712	1,670	1,690	1,685	1,691
Residues in the mature chain	1,642	1,676	1,652	1,652	1,659	1,670
Residues in collagenous domain	1,413	1,449	1,410	1,421	1,430	1,417
Residues in NC1 domain	229	227	232	231	229	228
Interruptions in the collagenous domain	21	23	23	26	22	25
Cysteine residues (NC1 domain)	12	12	12	12	12	12
Cysteine residues (7S + collagenous domain)	8	9	12	20	8	9

Chromosomal localization, number of residues in different domains, number of interruptions in collagenous domain, and total number of cysteine residues for each chain is indicated. Data adapted from (Zhou and Reeders, 1996).