Commentary

The Role of Type IV Collagen and Basement Membranes in Cancer Progression and Metastasis

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The basement membrane acts as a barrier separating the epithelium from the surrounding stroma. While the structural role of the basement membrane in defining tissue architecture and compartmentalization has been appreciated for decades, its dynamic role in the regulation of cell behavior and recruitment of signaling pathways is only now emerging.¹ The basement membrane further defines the tumor microenvironment and provides significant host-derived regulatory signals during progression of tumor growth and metastasis. The major component of the basement membrane is known to be type IV collagen.¹ Nevertheless, a precise role for type IV collagen in its assembled suprastructural form versus its unassembled/degraded form is primarily unknown.

In this issue of *The American Journal of Pathology*, Ikeda and colleagues² demonstrate the effect of promoter hypermethylation on the expression levels of COL4A5/COL4A6 collagen genes. They suggest that such hypermethylation leads to diminished levels of $\alpha 5(\text{IV})$ and $\alpha 6(\text{IV})$ collagen in colorectal cancer tissue and colon cancer cells. The normal production and assembly of the basement membrane is disrupted in this malignant process. This data is consistent with evidence emerging from recent studies examining different cancer tissues, including prostate cancer, using antibodies generated in the Sado Ninomiya laboratory.^{3–8} However, a lack of free access to these antibodies to all investigators has limited independent evaluation.

In vitro studies demonstrate that the expression of $\alpha 5(\text{IV})$ and $\alpha 6(\text{IV})$ collagen is down-regulated in association with hypermethylation of the bidirectional promoter of COL4A5/COL4A6 genes in colon cancer cell lines.² These results suggest that normal assembly and organization of the basement membrane is disrupted in cancer progression.² In an earlier study, Tanaka and colleagues⁹ analyzed the expression of the $\alpha 1(\text{IV})$ to $\alpha 6(\text{IV})$

collagen chain assembly by immunohistochemical analysis in colorectal cancer tissue, revealing normal expression of $\alpha 1(\text{IV})/\alpha 2(\text{IV})$ and $\alpha 5(\text{IV})/\alpha 6(\text{IV})$ chains but lost expression of $\alpha 5(\text{IV})/\alpha 6(\text{IV})$ chains upon cancer invasion. These authors suggested that loss of $\alpha 5(\text{IV})/\alpha 6(\text{IV})$ expression in the invasive cancer may be attributable to remodeling of basement membrane assembly, which in turn may be regulated by gene expression. The present article by Ikeda and colleagues² now further demonstrates that such loss may be at the gene expression level.

Basement Membranes and Type IV Collagen

Basement membranes are dynamic thin sheet-like structures of extracellular matrix that provide a supporting structure on which epithelial and endothelial cells reside. Under specialized conditions, they can act as filters with selective permeability, as in the kidney glomerulus, or as a highly specialized substrate for cellular differentiation and gene expression in other tissues. Several studies support the importance of maintaining normal architecture of the basement membrane and that its disruption is associated with different stages of invasive carcinomas.^{10,11} During malignant transformation normal tissue architecture is disrupted by factors produced and secreted by cancer cells. Matrix metalloproteinases, for example, are up-regulated in cancer cells and other cells associated with the tumor microenvironment, and many studies have shown matrix metalloproteinases to be responsible for loss of basement membrane architecture.

Basement membranes are composed of highly complex arrays of ~50 glycoproteins.¹ Type IV collagen,

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along with laminin, plays an important role in cell adhesion, migration, differentiation, and growth.¹² In this regard, degradation of type IV collagen can occur under both physiological and pathological conditions.¹ Type IV collagen degradation products also play an important role during angiogenesis,³ tissue remodeling, and cancer progression.^{4,5} Importantly, the significance of basement membrane type IV collagen synthesis during angiogenesis was determined from studies using inhibitors such as GPA 1734, D609, *cis*-hydroxyproline, and β -aminopropionitrile, which inhibit collagen biosynthesis, prevent endothelial tube formation and tumor growth.¹

Type IV collagen is present ubiquitously in all basement membranes. Each type IV collagen α -chain consists of three domains: an N-terminal 7S domain, a middle triple-helical domain, and a C-terminal globular noncollagenous (NC1) domain.¹ It is speculated that the six α -chains of type IV collagen self assemble to form predominantly three sets of triple-helical molecules that self associate via their NC1 domains and their middle triple-helical regions to form spider web-like scaffolds that interact with the laminin network and form a basic basement membrane scaffold.¹

Type IV Collagen Expression and Hypermethylation

The triple helical α -chains of type IV collagen are encoded by six distinct genes from $\alpha 1(IV)$ to $\alpha 6(IV)$, which are evolutionarily conserved and found in most organisms.^{1,13,14} A unique feature of these six genes is that they are located on three different chromosomes and are paired in a head-to-head manner: COL4A1-COL4A2 on chromosome 13q34,² COL4A3-COL4A4 on chromosome 2q36,² and COL4A5-COL4A6 on chromosome Xq22.² The expression of $\alpha 1(IV)$ and $\alpha 2(IV)$ chains are ubiquitous, but the other isoforms exhibit restricted distribution, such as $\alpha 5(IV)/\alpha 6(IV)$ in the basement membrane of mammary duct and lobule, epidermis, prostate gland, smooth muscle cells, and epithelium of alimentary tract.² The physiological importance of appropriate expression of the COL4A5 and COL4A6 genes is evident from studies relating to Alport syndrome. In this hereditary disease, mutations of either the COL4A3, COL4A4, or COL4A5 gene results in the lack of $\alpha 3(IV)$, $\alpha 4(IV)$, or $\alpha 5(IV)$ collagen-containing protomers in the glomerular and cochlear basement membranes, leading to renal failure and sensorineural deafness in these individuals.¹⁵

In this issue of *The American Journal of Pathology*, Ikeda and colleagues² show that the normal production and assembly of the basement membrane is disrupted during malignant cancer progression, and they further imply that $\alpha 5(IV)/\alpha 6(IV)$ chains of type IV collagen might provide protective effects in determining the rate of cancer progression.¹ In support of this, studies from Nakano and colleagues¹⁶ have demonstrated by *in situ* hybridization and immunohistochemistry that assembly of $\alpha 5(IV)/\alpha 6(IV)$ is defective in the invasive stages of breast cancer.

The expression and the gene regulation of $\alpha 5(IV)/\alpha 6(IV)$ chains are not fully understood, but recent studies suggest that the bifunctional promoter of these genes can function in an independent manner depending on the cytokine influence.¹⁷ The expression of α -chains of type IV collagen is also influenced by certain growth factors that significantly enhance the expression of $\alpha 5(IV)$, but not $\alpha 6(IV)$ genes in glomerular endothelial and mesangial cells, whereas $\alpha 6(IV)$ gene expression is enhanced in tubular epithelial cells as compared to the $\alpha 5(IV)$ genes. Silencing of tumor suppressor genes by methylation of genes in promoter region has been a major focus in identifying causative reasons for progression of human cancers,¹⁸ and hypermethylation has now been shown to be important in collagen IV gene expression. The presented studies also suggest that the proximal bifunctional promoters are critical in regulating the expression of these α -chains in a cell-specific manner.¹⁷ In mice engineered to express lacZ from the COL4A5/COL4A6 promoter, the specialized pattern of COL4A5/A6 expression was demonstrated to be due to interaction of *cis*-acting elements with the proximal promoter. In these mice, lacZ staining was only observed in certain cell layers of the upper gastrointestinal tract but not in the kidneys, lungs, skin, or other sites, the same sites where type IV collagen $\alpha 5$ and $\alpha 6$ chains have been localized by antibody binding.¹⁷ For example, it has been shown that the laminin 5 gene, another basement membrane component, is down-regulated in prostate cancer tissue showing invasive cancer.¹⁹

Collectively, the studies reported by Ikeda and colleagues,² support the notion that loss of critical type IV collagen chains in the setting of colon cancer, and possibly other cancers, can potentially provide a permissive microenvironment for invasiveness phenotype of cancer. Such alterations in selective type IV collagen expression are likely regulated at the gene level by hypermethylation events. How such events influence cancer progression still needs to be evaluated.

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