Monoclonal Collagen XVII Antibody

Antibody against Human Collagen XVII, Clone NC16A-3

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Technology

Collagen XVII (BP180) is a transmembrane protein of the cutaneous basement membrane zone that plays an important role in epidermal-dermal adhesion. This notion is supported by its pathogenetic relevance as autoantigen in several autoimmune bullous disorders and as mutated protein in certain forms of inherited epidermolysis bullosa. Collagen XVII migrates at 180 kDa in keratinocyte extracts. Proteolytic cleavage of the ectodomain results in a soluble 120 kDa form. This antibody is produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. Purified recombinant NC16a-domain of human collagen XVII was used as immunogen. This clone (mouse IgG1 isotype) has been evaluated for specificity using indirect immunofluorescence on human skin and western blot with extracts and conditioned medium of cultured keratinocytes. By PepSpot-analysis, the epitope recognized by this clone was localized to amino acids 545 to 557 of collagen XVII.

Innovation
- May be the first commercially available monoclonal antibody against human collagen XVII
- High specificity, homogeneity and availability in unlimited amounts
- Recognizes native and denatured collagen XVII by a wide range of biochemical methods
- Binds to 180 kDa collagen XVII in keratinocyte extracts AND to the shed 120 kDa ectodomain in keratinocyte medium or biological fluids which is in contrast to previously published

Application
- Indirect immunofluorescence
- Immunohistochemistry on formalin-fixed paraffin-embedded human skin sections
- ELISA | Western Blot | Immunoprecipitation
- Monitoring of collagen XVII-levels in biological fluids like blister fluids

Market Potential

Most applicable for the diagnosis of inherited blistering disorders where collagen XVII may be absent or reduced in the skin. In tumor specimens it may be used as a marker for basement membrane invasion. At present, there aren’t any monoclonal collagen XVII antibodies commercially available, indicating a vast market potential also for experimental basement membrane research.

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Monoclonal antibodies against human collagen XVII (clone NC16a-3)

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Introduction

- Collagen XVII (BP180) is a hemidesmosomal transmembrane protein which mediates epidermal-matrix adhesion. Its 120 kDa-ectodomain is constitutively shed from the cell surface by ADAMs (a disintegrin and metalloproteinase).
- The NC16a-domain of collagen XVII is the major autoantigen in bullous pemphigoid.
- Mutations in the collagen XVII gene (COL17A1) lead to altered or reduced expression of collagen XVII causing junctional epidermolysis bullosa, a severe inherited skin-blistering disorder.
- In addition to its function in epithelial-matrix adhesion, collagen XVII plays a role in cell migration and differentiation.
- The NC16a-domain of collagen XVII is the membrane-adjacent domain and represents the major target in bullous pemphigoid.

Results

mAb NC16a-3 detects native and unfolded collagen XVII

Cryosections of normal human skin and from patients with inherited skin-blistering disorders and absence of collagen XVII or laminin 5 were stained with mAb NC16a-3 and a rabbit-anti-laminin 5 antibody.

NC16a-3 (diluted 1: 100,000) detects collagen XVII and its 120kDa shed ectodomain by western blotting with keratinocyte extract (E) and keratinocyte medium (M), respectively.

The epitope of mAb NC16a-3 is mapped to amino acids 545-557 of the NC16a-domain

PepSpot analysis using 35 overlapping 13-mer peptides revealed that the epitope of mAb NC16a-3 spans amino acids 545-557.

mAb NC16a-3 immunoprecipitates the collagen XVII-ectodomain from blister fluids

mAb NC16a-3 efficiently immunoprecipitates the 120kDa shed ectodomain of collagen XVII from blister fluids. Immunoprecipitation was carried out by incubation of mAb NC16a-3 with blister fluids from 10 BP patients and protein A sepharose. Eluates were blotted onto nitrocellulose and incubated with polyclonal rabbit-anti-NC16a-antibody. M = conditioned keratinocyte medium as positive control.

mAb NC16a-3 stains the basement membrane by immunohistochemistry of paraffin-embedded skin sections

Formalin fixed, paraffin-embedded sections of human skin were pretreated with target retrieval pH9 (DAKO) and incubated with mAb NC16a-3. The antibody shows a linear staining of the basement membrane zone and a pericellular staining of the basal keratinocytes. In addition, epithelial tumors show enhanced expression of collagen XVII. a and c, basal cell carcinoma; b, Bowens disease; d, squamous cell carcinoma.

Conclusions

- mAb NC16a-3 is a mouse IgG1-antibody directed against amino acids 545-557 of the collagen XVII-ectodomain (NC16a-domain).
- The antibody binds specifically to collagen XVII in cryo-fixed and paraffin-embedded skin sections. It may therefore be used for the diagnosis of inherited blistering disorders. Furthermore, collagen XVII staining may facilitate the diagnosis of invasive tumors by visualization of the basement membrane.
- mAb NC16a-3 detect both, full-length collagen XVII and the 120 kDa shed ectodomain by western blot with cultured keratinocytes. The antibody may be diluted up to 1: 500.000 by western blot analysis indicating very high affinity to denatured collagen XVII. Therefore mAb NC16a-3 represents a very useful tool for basement membrane research.

References: