antibodies

MDPI

Article

Two Highly Specific Mouse Monoclonal Antibodies to the Putative C-Telopeptide of Human Collagen XIα1, a Cancer Biomarker

Marcos García-Ocaña [1], Lorea Legazpi-Olabide [2], Sandra Rodríguez-Rodero [2], Paula Rodríguez-Folgueira [3], Iván Fernández-Vega [4,5], Marcos Ladreda-Mochales [6], Juan R. de los Toyos [2,3,7,*] and Luis J. García-Flórez [2,7,8,9,*]

1 Biomedical and Biotechnological Tests Unit, Scientific and Technical Services, University of Oviedo, 33006 Oviedo, Spain; garciaomarcos@uniovi.es
2 Abdominal Surgical Pathology Group, Health Research Institute of the Principality of Asturias and Foundation for Biohealth Research and Innovation of the Principality of Asturias "ISPA-FINBA", 33011 Oviedo, Spain; llegazpi2001@yahoo.es (L.L.-O.); rodriguezrodero@gmail.com (S.R.-R.)
3 Immunology Area, Functional Biology Department, University of Oviedo, 33006 Oviedo, Spain; uo273422@uniovi.es
4 Biobank of the Principality of Asturias "BioPA", Health Research Institute of the Principality of Asturias and Foundation for Biohealth Research and Innovation of the Principality of Asturias "ISPA-FINBA", 33011 Oviedo, Spain; fernandezvivan@uniovi.es
5 Pathological Anatomy Area, Surgery and Medical-Surgical Specialties Department, University of Oviedo, 33006 Oviedo, Spain
6 Startquake, S.L., IMPULSA Building Gijón Scientific and Technological Park, 33203 Gijón, Spain; marcos@eternadx.com
7 The University Institute of Oncology of Asturias "IUOPA", University of Oviedo, 33006 Oviedo, Spain
8 Surgery Area, Surgery and Medical-Surgical Specialties Department, University of Oviedo, 33006 Oviedo, Spain
9 General Surgery Service, Central University Hospital of Asturias, 33011 Oviedo, Spain
* Correspondence: jrtoyos@uniovi.es (J.R.d.l.T.); garciafluis@uniovi.es (L.J.G.-F.)

Abstract

Background: Collagen XIα1, encoded by the *COL11A1* gene, is a minor fibrillar collagen that is overexpressed in various human cancers, in which its presence correlates with tumor aggressiveness and progression. Methods: In this study, we developed two novel mouse monoclonal antibodies (mAbs)—anti-colXIα1 clone 3 and anti-colXIα1 clone 9—that target the putative C-telopeptide of human collagen XIα1. These antibodies target the RRHTEGMQA sequence, a unique nine-amino-acid stretch within the putative C-telopeptide of human collagen XIα1. Results: Corresponding to nearly identical V(D)J gene segments and complementarity-determining regions (CDRs), the antibodies specifically bound the RRHTEGMQA epitope in ELISAs but did not react with the C-propeptide. This specificity was further confirmed with the purified anti-colXIα1 clone 9 mAb, which demonstrated strong reactivity against recombinant proteins containing the RRHTEGMQA sequence in both ELISAs and Western blot assays. This sequence seems to behave as a linear B-cell neoepitope, in which the RRHT motif is crucial for epitope recognition. Otherwise, no immunodetections were observed, either in cultures and lysates from the *COL11A1*-highly expressing A204 human cell line or on tissue sections from specimens of human pancreatic ductal adenocarcinoma (PDAC), with strong desmoplastic reactions. Conclusions: Given the lack of precise knowledge of the characteristics of the putative C-telopeptide of human collagen XIα1, the presented antibodies could enhance our understanding of the processing of human procollagen XIα1 and contribute to better characterization of the tumor microenvironment of *COL11A1*-expressing cancers.

Check for updates

Academic Editor: Itai Benhar

Received: 21 January 2026
Revised: 12 February 2026
Accepted: 20 February 2026
Published: 25 February 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

Keywords: mouse monoclonal antibodies; *COL11A1*; human collagen XIα1; putative C-telopeptide

1. Introduction

The *COL11A1* human gene codes for the α1 chain of human procollagen XI and mature collagen XI, an extracellular minor fibrillar collagen. (Pro)collagen XIα1 is highly synthesized in diverse human cancers, and its expression is correlated with tumor aggressiveness and progression [1–6]. Together with the *INHBA* and *THBS2* genes, the *COL11A1* gene was initially correlated with a multicancer invasion- and metastasis-associated gene expression signature [1]. Later on, this gene was also identified as a member of a pancancer signature present in late-stage aggressive cancers [4]. As components of the extracellular matrix, collagens remodel the tumor microenvironment. Matrix stiffening and collagen fiber alignment—in which collagen XIα1 is thought to be involved—have been shown to promote cancer cell migration [5]. Conversely, knockdown of the *COL11A1* gene significantly diminishes the invasive potential of cancer cells [4].

COL11A1/(pro)collagen XIα1 is mainly expressed by a subset of myofibroblastic cancer-associated fibroblasts (CAFs), which are also specifically characterized by their surface expression of leucine-rich repeat-containing 15 (LRRC15) protein and integrin alpha-11 (ITGA11), as well as their secretion of matrix metalloproteinase-11 (MMP-11, stromelysin 3). These *COL11A1*+ myofibroblasts are prominent in the desmoplastic reaction of human invasive carcinomas [7–11].

Some well-established human cancer cell lines also express high levels of *COL11A1* mRNA/(pro)collagen XIα1 [12] and are useful as models to track the expression and biological significance of this extracellular matrix component.

The canonical sequence of the P12107-1 Name A isoform of human procollagen XIα1 consists of 1806 amino acid residues and has an estimated molecular weight of 181 kDa [13]. Based on comparison with other better-characterized collagens, this procollagen is expected to be processed by extracellular proteinases upon secretion, excising the N- and C-terminal propeptides [14]. The 21 C-terminal residues of the processed mature extracellular collagen XIα1—(1543) IQPLPILSSKKTRRHTEGMQA (1563)—would then comprise the putative nonhelical C-telopeptide [13].

Antibodies to the N-propeptide and C-propeptide of human procollagen XIα1 are available. Some antibodies are assumed to detect collagen XIα1, but the epitope and/or immunogen used for their generation has not been described. Neither their fine specificity in relation to other collagens nor their immunoreactivity in human tissues has been assessed in detail.

Monoclonal antibodies to the C-telopeptide of human collagen XIα1 could help shed light on the actual processing of human procollagen XIα1 and lead to a better characterization of *COL11A1*-expressing tumors and the extracellular matrix microenvironment. In addition, the tuned derivatives of these antibodies—either naked, as antibody–drug conjugates (ADCs), or as components of targeted immunovesicles (liposomes and exosomes)/immunonanoparticles—could be employed as therapeutic weapons to combat the progression of *COL11A1*-expressing tumors. However, to date, no antibodies to this region have been generated.

In this study, we developed two novel mouse monoclonal antibodies (mAbs)—anti-colXIα1 clone 3 and anti-colXIα1 clone 9—to the putative C-telopeptide of human collagen XIα1 and assessed their reactivity and genetic features.

2. Materials and Methods

2.1. Human Collagen XIα1 and C-Propeptide Recombinant Forms

The putative extracellular processed form of human collagen XIα1 (Figure 1), with an added 6xHis head, recombinantly expressed in CHO cells, was provided by GenScript (Piscataway, NJ 08854, USA). It has a theoretical molecular weight of 98.37 kDa.

U9642210G0-1_Collagen XIalfa1

Protein sequence: 1077 aa

MGWSCIILFLVATATGVHSHHHHHHQEAQAQAILQQARIALRGPPGPMGLTGRPGPVGGPGSSGAKGESGDPGPQGPRGVQGPPGPTGKPGKRGR
PGADGGRGMPGEPGAKGDRGFDGLPGLPGDKGHRGERGPQGPPGPPGDDGMRGEDGEIGPRGLPGEAGPRGLLGPRGTPGAPGQPGMAGVD
GPPGPKGNMGPQGEPGPPGQQGNPGPQGLPGPQGPIGPPGEKGPQGKPGLAGLPGADGPPGHPGKEGQSGEKGALGPPGPQGPIGYPGPRGV
KGADGVRGLKGSKGEKGEDGFPGFKGDMGLKGDRGEVGQIGPRGEDGPEGPKGRAGPTGDPGPSGQAGEKGKLGVPGLPGYPGRQGPKGSTGF
PGFPGANGEKGARGVAGKPGPRGQRGPTGPRGSRGARGPTGKPGPKGTSGGDGPPGPPGERGPQGPQGPVGFPGPKGPPGPPGKDGLPGHPG
QRGETGFQGKTGPPGPGGVVGPQGPTGETGPIGERGHPGPPGPPGEQGLPGAAGKEGAKGDPGPQGISGKDGPAGLRGFPGERGLPGAQGAPG
LKGGEGPQGPPGPVGSPGERGSAGTAGPIGLPGRPGPQGPPGPAGEKGAPGEKGPQGPAGRDGVQGPVGLPGPAGPAGSPGEDGDKGEIGEPG
QKGSKGDKGENGPPGPPGLQGPVGAPGIAGGDGEPGPRGQQGMFGQKGDEGARGFPGPPGPIGLQGLPGPPGEKGENGDVGPMGPPGPPGPR
GPQGPNGADGPQGPPGSVGSVGGVGEKGEPGEAGNPGPPGEAGVGGPKGERGEKGEAGPPGAAGPPGAKGPPGDDGPKGNPGPVGFPGDPG
PPGEPGPAGQDGVGGDKGEDGDPGQPGPPGPSGEAGPPGPPGKRGPPGAAGAEGRQGEKGAKGEAGAEGPPGKTGPVGPQGPAGKPGPEGLR
GIPGPVGEQGLPGAAGQDGPPGPMGPPGLPGLKGDPGSKGEKGHPGLIGLIGPPGEQGEKGDRGLPGTQGSPGAKGDGGIPGPAGPLGPPGPPGL
PGPQGPKGNKGSTGPAGQKGDSGLPGPPGSPGPPGEVIQPLPILSSKKTRRHTEGMQA*

Figure 1. The amino acid sequence of the putative extracellular processed form of human collagen XIα1 (residues 512 to 1563 of the P12107-1 A isoform), recombinantly expressed in CHO cells (provided by GenScript). It encompasses the C-terminal telopeptide—(1543) IQPLPILSSKKTRRHTEGMQA (1563)—sequence. It is preceded by the MGWSCIILFLVATATGVHS signal peptide and a 6xHis head.

Similarly, the putative C-propeptide, comprising 243 amino acid residues (Figure 2) and with a theoretical molecular weight of 27.45 kDa, was also recombinantly expressed in CHO cells using GenScript.

Expression Sequence:

MGWSCIILFLVATATGVHSDADDNILDYSDGMEEIFGSLNSLKQDIEHMKFPMGTQTNPARTCKDLQLSHP
DFPDGEYWIDPNQGCSGDSFKVYCNFTSGGETCIYPDKKSEGVRISSWPKEKPGSWFSEFKRGKLLSYLDVE
GNSINMVQMTFLKLLTASARQNFTYHCHQSAAWYDVSSGSYDKALRFLGSNDEEMSYDNNPFIKTLYDGC
ASRKGYEKTVIEINTPKIDQVPIVDVM INDFGDQNQKFGFEVGPVCFLG*

Figure 2. The amino acid sequence of the putative C-propeptide, from human procollagen XIα1 (residues 1564 to 1806 of the P12107-1 A isoform), recombinantly expressed in CHO cells (provided by GenScript). It is preceded by the MGWSCIILFLVATATGVHS signal peptide.

The COL11A1 Fusion Protein, with an N-terminal GST tag, from ChromoTek GmbH and Proteintech, Germany (Cat. No. Ag16509), was recombinantly expressed in *Escherichia coli*. After removal of the GST tag (Ag37791), the first 19 N-terminal amino acid residues PLPILSSKKTRRHTEGMQA corresponded to the putative C-telopeptide (Figure 3). This product has a theoretical molecular weight of 29.6 kDa.

Peptide Sequence:
PLPILSSKKTRRHTEGMQADADDNILDYSDGMEEIFGSLNSLKQDIEHMKFPM
GTQTNPARTCKDLQLSHPDFPDGEYWIDPNQGCSGDSFKVYCNFTSGGETCIYP
DKKSEGVRISSWPKEKPGSWFSEFKRGKLLSYLDVEGNSINMVQMTFLKLLTAS
ARQNFTYHCHQSAAWYDVSSGSYDKALRFLGSNDEEMSYDNNPFIKTLYDGC
ASRKGYEKTVIEINTPKIDQVPIVDVMINDFGDQNQKFGFEVGPVCFLG

Figure 3. The amino acid sequence of the COL11A1 Fusion Protein (residues 1545 to 1806 of the P12107-1 A isoform), recombinantly expressed in *Escherichia coli*, after the removal of an N-terminal GST tag (provided by Proteintech). The first 19 N-terminal (1545) PLPILSSKKTRRHTEGMQA (1563) amino acid residues are part of the putative C-telopeptide. The next 243 amino acid residues (1564 to 1806) constitute the C-propeptide.

2.2. Mouse Immunization and Hybridoma Generation

Peptide conjugates KLH-Cys-GG-RRHTEGMQA and KLH-Cys-GG-EGMQADADD, corresponding to different amino acid stretches of the human procollagen XIα1 around the C-telopeptide, were provided by Abyntek Biopharma S.L. (Zamudio, Bizkaia, Spain). Cysteine and glycine residues were added to ensure correct linking to the carrier protein, as previously reported [15]. Upon reconstitution in sterile water, B Braun, and saline for injections, an emulsion was obtained with the Sigma Adjuvant System (Sigma-Aldrich, Cat. No. S6322) (St. Louis, MO, USA) for immunization purposes, following the manufacturer's instructions.

Female 6-week-old DBA/1J mice were obtained from The Jackson Laboratory through Charles River Laboratories S.A., Spain, and female 4-week-old BALB/c mice were obtained from Charles River Laboratories S.A., Spain. The mice were housed in the facilities of the Bioterio of the University of Oviedo under conventional conditions. They were handled following the Guidelines of the Experimental Animal Ethics Committee of the University of Oviedo, after the approval of animal experimental procedures by the Government of the Principality of Asturias, Spain, Resolutions *PROAE 62/2019* and *PROAE 4/2022.*

Mice were i.p. injected with 200 µg of conjugate/500 µL of emulsion, at least 15 days apart. Once hyperimmunized, they were euthanized via cervical dislocation for hybridoma generation. Spleens were aseptically removed and gently homogenized for fusion with Sp2/0 mouse myeloma cells using standard methods [16].

Hybridoma supernatants, in RPMI medium/hypoxanthine–aminopterin–thymidine (HAT)/20% fetal bovine serum (FBS), were primarily screened using an indirect ELISA against KLH, mechanical lysates from A204 and A549 cell cultures, and the human collagen XIα1 recombinant form (Figure 1).

A 1:1000 dilution of anti-mouse γ-chain-HRPO (Sigma-Aldrich, Cat. No. A3673) in 10 mM phosphate-buffered saline (PBS)–1% bovine serum albumin (BSA)–0.1% Tween 20 was applied to identify mouse IgG-containing supernatants.

The selected antibody-secreting hybridomas were subcloned twice to ensure antibody monoclonality. Antibody sub-isotyping was conducted using the IsoStrip™ Mouse Monoclonal Antibody Isotyping Kit (11493027001 Roche).

For the purified antibody preparations, subclones were progressively adapted to grow in RPMI Medium–20% FBS and, subsequently, in protein-free and serum-free CD Hybridoma Gibco™ Medium (ThermoFisher Scientific, Cat. No. 11279023)–20% FBS. Finally, they were weaned from FBS.

Cultures were assessed to be free of *Mycoplasma* using the MycoStrip™ Mycoplasma Detection Kit (InvivoGen, Cat. No. rep-mys-10), following the manufacturer's instructions.

The monoclonal antibodies were purified using an Amersham Biosciences ÄKTA Purifier 10 FPLC System w/ UPC-900 and 1 mL MabCaptureC™ MiniChrom Columns (ThermoFisher Scientific, Cat. No. 5943662001) (Waltham, MA, USA), following the manufacturer's instructions. They were then dialyzed against 10 mM PBS (pH 7.4), concentrated via centrifugation in 10K concentrators and, finally, filtered through sterile 0.25 µm filters.

2.3. Isotype-Matched Negative Control mAb

Anti-pneumolysin IgG1, kappa PLY-7 mAb [17] served as the isotype-matched negative control.

2.4. Cell Cultures and Lysates

Human rhabdomyosarcoma A204 (no. HTB-82), large-cell lung carcinoma NCI-H661 (no. HTB-183), and alveolar lung carcinoma A549 (no. CCL-185) cell lines were obtained from the American Type Culture Collection (ATCC) and cultured in DMEM supplemented

with sodium pyruvate, L-glutamine, non-essential amino acids, 10% FBS, ascorbate 2-phosphate (37.5 μg/mL) (Sigma-Aldrich, Cat. No. A8960), and 10 ng/mL of recombinant TGF-β1 (PeproTech, Cat. No. 100-21C) in a humidified atmosphere of 5% CO_2 in air at 37 °C. These cell lines have been previously reported to express significantly different levels of *COL11A1* mRNA [12].

The cells were cultured for at least 15 days in T-25 and T-75 flasks (Sarstedt, Cat. No. 83.3910.302 and 83.3911.302, respectively) (Nümbrecht, North Rhine-Westphalia, Germany). Passages and cell collections were conducted via trypsinization. Different harvests from each cell culture condition were pooled.

For immunocytochemistry (ICC), cells were cultured in four-well culture slides (BD Falcon™, ref. 354114, or Nunc™ Lab-Tek™ II CC2™) (ThermoFisher Scientific, Cat. No. 154917PK, or Sarstedt, Cat. No. 94.6170.402) for three to five days.

After the removal of spent media, the flasks were washed with 10 mM of cold PBS–0.1% sodium azide (PBS–SA) to generate cell lysates. Cell cultures were then vigorously collected in cold PBS–SA or a hot SDS-PAGE sample buffer with the help of cell scrapers. The cell lysates were frozen and thawed several times.

2.5. ELISA for Antibody Reactivity Screening and Assessment

Flat-bottom 96-well MaxiSorp Nunc-Immuno plates (ThermoFisher Scientific, Cat. No. 439454) were coated with 1 μg/100 μL well of the human collagen XIα1 recombinant form, the human collagen XIα1 C-propeptide recombinant form, the COL11A1 Fusion Protein with no GST tag (Ag37791), the peptide conjugates used as immunogens, or 10 μg/100 μL well of mechanical cell lysates, in PBS–SA at pH 7.3 for 6 h at 37 °C. They were then blocked with 200 μL/well of PBS–1% BSA–SA for 1 h at 37 °C and left overnight at 4 °C. After being washed, the wells were successively incubated with 100 μL/well of crude hybridoma supernatants or with 1 μg/100 μL well of purified monoclonal antibody in PBS-1% BSA–SA (for 2 h at 37 °C); 100 μL per well of a 1:1000 dilution of anti-mouse γ-chain-HRPO (Sigma-Aldrich, Cat, No. A3673) in PBS–1% BSA–0.1% Tween 20 (for 1 h at 37 °C); and 100 μL per well of ready-to-use supersensitive TMB solution for the ELISA (Sigma-Aldrich, Cat, No. T4444) (for 5 min at 37 °C). Color development was stopped by adding 100 μL per well of 2 M H_2SO_4. Plates were read at 450 nm with a Synergy LX Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). All determinations were made in triplicate and averaged. The data were analyzed using the BioTek Gen5 software. Optical density data were blanked by subtracting blanks (culture media or PBS–1% BSA–SA) from the readings.

The plates were washed three times with 200 μL/well of PBS–0.1% Tween-20 between incubations.

2.6. ELISA Competition Tests with Soluble Peptides

ELISA competition tests with soluble peptides were performed essentially as already described [18]. Immunograde, ≥95% pure synthetic N-acetylated RRHTEGMQA, EGMQADADD, and (450)KRTISIWGT(458) from pneumolysin—as a scrambled control—peptides were supplied by Abyntek Biopharma S.L. (Zamudio, Bizkaia, Spain).

ELISA plates were coated with the human collagen XIα1 recombinant form, as described above. The reactivity of each supernatant was titered in ELISAs. An assessed dilution of each supernatant, which rendered a reliable absorbance signal, was chosen as a positive control.

For competition tests, the supernatant dilutions were mixed at equal volumes with 1:10 serial dilutions of the synthetic peptide in PBS–1% BSA–SA (the final peptide concentration in the wells was from 2.5×10^{-1} to 2.5×10^{6} ng/mL, as shown in Figure 4), then preincubated for 2 h at 37 °C and overnight at 4 °C. The next day, the mixtures were added

to the ELISA coated wells and incubated for 2 h at 37 °C. The rest of the ELISA continued as described above. No peptide was added in the blanks. The inhibitory activity of any blocking peptide sample was estimated from its color reading in relation to the absorbance of the positive controls.

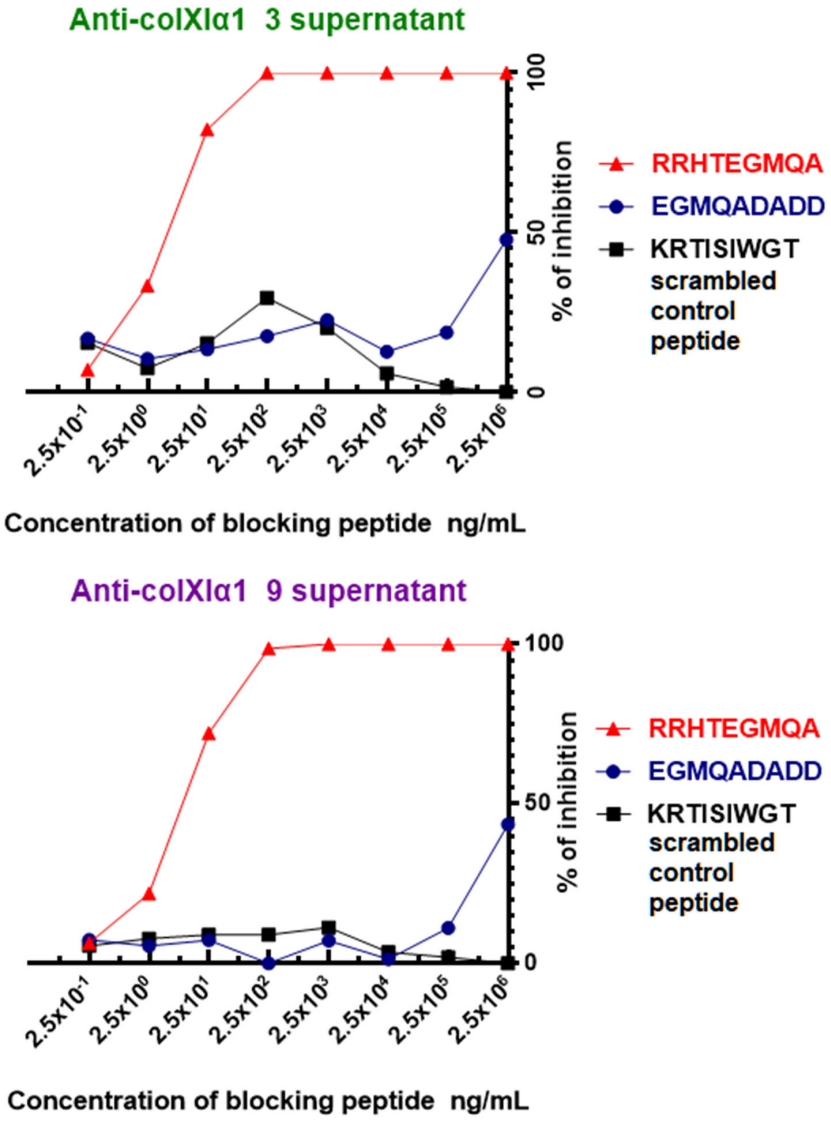


Figure 4. ELISA blocking of recognition of recombinant collagen XIα1 with soluble-free N-acetylated peptides. Figure drawn using GraphPad Prism Version 10.0.3 (275) for Windows (GraphPad Software, Boston, MA, USA).

2.7. SDS-PAGE and Western Blot Assays

Human Immunization-Grade Type XI Collagen was purchased from Chondrex (Catalog # 1085).

Purified protein concentrations were estimated using absorbance at 280 nm with a UV-1280 UV–vis spectrophotometer (Shimadzu, Kyoto, Japan).

A total of 15 µg/lane of recombinant protein or 50 µg/lane or 40 µL/lane of whole-cell lysates and 10 µL/lane of PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa (ThermoFisher Scientific, Cat. No. 26619), was subjected to 12, 10, or 6% polyacrylamide SDS-PAGE under reducing conditions and, subsequently, electrotransferred onto an Immobilon®-NC Transfer Membrane, with a 0.45 µm pore size (Sigma-Aldrich, Cat, No. HATF00010), using the Mini-PROTEAN® Tetra Cell (2-gel) and Tetra Blotting Module (BIO-RAD, Hercules, CA, USA).

Some cell lysates were also generated by scraping cell cultures in the presence of hot sample buffer.

After blocking overnight at 4 °C in PBS–3% BSA–SA, the membranes were probed with 10 mL of crude hybridoma supernatants or with 10 µg of purified mAb in 10 mL of PBS–1% BSA–SA with gentle rocking for 2 h at room temperature. After several washing steps with PBS–0.1% Tween-20 and PBS, the blots were incubated with 10 mL of a 1:4000 dilution of anti-mouse γ chain-specific HRPO conjugated (Sigma-Aldrich, Cat, No. A3673) in PBS–1% BSA–0.1% Tween-20 for 2 h, as described above, and finally developed with 1-Step™ Ultra TMB Blotting Solution (ThermoFisher Scientific, Cat. No. 37574), following the manufacturer's instructions.

2.8. PEP-FOLD4 Peptide Structure Predictions

Peptide sequences were submitted to the PEP-FOLD4 *online* server under the current default conditions to gain insight into the potential conformation of the C-telopeptide in the assayed antigenic preparations and support our observations [19].

2.9. V Gene Sequencing of Hybridomas

The whole heavy and light chains of mAbs were sequenced using whole-transcriptome shotgun sequencing (RNA-Seq), Absolute Antibody Ltd., Oxfordshire, United Kingdom.

Nucleotide sequences were analyzed using IMGT/V-QUEST (https://www.imgt.org/IMGTindex/V-QUEST.php; accessed on 9 January 2024) [20].

2.10. Immunocytochemistry (ICC)

ICC procedures were performed by personnel of the Molecular Histopathology Unit in Animal Models of Cancer (IUOPA).

Cells were fixed in 4% formaldehyde for 15 min in the chamber slide. Primary antibodies were applied, as described in Table 1.

Table 1. Antibodies used in ICC analyses.

Primary Antibody (Species)	Protein Recognized	Source	Dilution	Incubation Conditions
Anti-colXIα1 clone 9 (mAb)	Human collagen XIα1	In-house	1:300 from 1 mg/mL	48 h at 4 °C
PLY-7 (mAb)	Pneumococcal pneumolysin	In-house	1:300 from 1 mg/mL	48 h at 4 °C
Vimentin antibody (V9) (mAb)	Human vimentin	Santa Cruz Biotechnology, Inc. (Dallas, TX 75220, USA) SC-6260	1:500	48 h at 4 °C

The primary antibody was omitted in the negative controls. Subsequently, slides were incubated with the EnVision FLEX HRP (DAKO K8000) system for 30 min at room temperature. The samples were then visualized with diaminobenzidine (DAB) for 30 s. Finally, the immunostained slides were dehydrated, mounted, studied under an Olympus BX61 Automatic Microscope, and photographed using the DP Controller (1.2.1.108)/Manager (1.2.1.107) software with the assistance of personnel of the Photonic Microscopy and Image Processing Unit of the Scientific and Technical Services (SCTs) of the University of Oviedo.

2.11. Immunohistochemistry (IHC)

For immunohistochemical techniques, specimens of pancreatic ductal adenocarcinoma (PDAC), with strong desmoplastic reactions, and normal pancreas counterparts from the

same patients were provided by the Principality of Asturias BioBank (PT23/077). The specimens were processed following standard operating procedures with the appropriate approval of the Ethical and Scientific Committees (Comité de Ética de la Investigación con Medicamentos del Principado de Asturias; reference: CEImPA 2022.462).

IHC procedures were performed by personnel of the BioBank. The DAKO Autostainer system was used.

The samples were fixed with 10% formaldehyde for 24 h and embedded in paraffin. Tissue sections (3 µm) were deparaffinized, rehydrated, and subjected to epitope retrieval using heat induction (HIER) at 95 °C for 20 min, at pH 9 and pH 6 (Agilent-DAKO, Glostrup, Denmark), in the Pre-Treatment Module, PT-LINK (DAKO). Endogenous peroxidase activity was blocked with the EnVision™ FLEX Peroxidase-Blocking Reagent (DM821) for 5 min. Subsequently, sections were first incubated with anti-colXIα1 clone 9 and PLY-7 (with a dilution of 1:100 from 1 mg/mL for both) for 30 min. Subsequently, the Dako EnVision® + Dual Link System-HRP (Agilent-DAKO) was applied. The samples were then stained using DAB chromogen as a substrate in Dako EnVision™ FLEX/HRP (Agilent-DAKO), counterstained with hematoxylin, dehydrated, and then mounted with permanent medium (Agilent-DAKO Mounting Medium, CS703). The sections were studied under a light microscope (Nikon—ECLIPSE C*i*) (Tokyo, Japan).

The negative controls were processed by omitting the primary antibody.

3. Results

3.1. mAb Reactivity

Upon immunization with the two designed immunogens, KLH-Cys-GG-RRHTEGMQA and KLH-Cys-GG-EGMQADADD, we sought to generate mAbs with the following abilities: besides interfering with proteinases, mAbs targeting the RRHTEGMQA sequence that could recognize the extracellular C-telopeptide and those targeting the EGMQADADD sequence that could recognize procollagen even before its processing. However, we were only successful in rescuing hybridomas from BALB/c mice after immunization with the KLH-Cys-GG-RRHTEGMQA immunogen.

The two mAbs described herein were generated from the same BALB/c mouse; both are of the IgG1 kappa subtype.

Table 2 shows the immunoreactivity of initial crude supernatants, in RPMI-HAT-20% FBS, from the two hybridomas against the components of the conjugates which were used as immunogens.

Table 2. Immunoreactivity against components of the immunogens.

ELISA [a]			
Antigen			
Supernatant/mAb	**Immunogen 1 KLH-C-GG-EGMQADADD**	**Immunogen 2 KLH-C-GG-RRHTEGMQA**	**KLH**
Anti-colXIα1 clone 3 #14/08/2023	-	3+	-
Anti-colXIα1 clone 9 #14/08/2023	2+	3+	-
Medium RPMI-HAT-20% FBS	-	-	-

[a] Color development was estimated by eye. # From supernatants in RPMI-HAT-20% FBS, with indication of collection date.

According to these results, the anti-colXIα1 clone 3 mAb seems to exclusively recognize the KLH-C-GG-RRHTEGMQA conjugate, while the anti-colXIα1 clone 9 mAb seems to also recognize the KLH-C-GG-EGMQADADD sequence, although with lower intensity.

Table 3 shows some other reactivity characteristics against different antigen preparations.

Table 3. Some other immunoreactivity characteristics.

Supernatant/mAb	ELISA [a]				Conventional Denaturing and Reducing Western Blot			
	Recombinant Collagen XIα1	Recombinant C-Propeptide	A204 Cell Lysate	A549 Cell Lysate	6% Gel	12% Gel	10% Gel	
					Purified Human Collagen XI	Recombinant C-Propeptide	A204 Cell Lysate	A549 Cell Lysate
Anti-colXIα1 clone 3	3.259	0.000	0.105	0.029	-	-	-	-
Anti-colXIα1 clone 9	3.519	0.000	0.055	0.064	-	-	-	-

[a] ELISA optical density scale of blanked data from 0 to 4.

In the ELISA, the anti-colXIα1 clone 3 and anti-colXIα1 clone 9 supernatants showed strong reactivity against the recombinant human collagen XIα1, but not against the recombinant C-propeptide or with cell lysates from the A204 and A549 cell lines. The human rhabdomyosarcoma A204 cell line, which expresses the highest recorded levels of *COL11A1* mRNA [12], was used as a positive control, while alveolar lung carcinoma A549 cells expressing very low levels of *COL11A1* mRNA served as a quasi-negative control. All the Western blots indicated in Table 3 were negative.

Blocking assays with soluble free N-acetylated peptides were performed to confirm the fine specificity of the two mAbs. As shown in Figure 4, there was a clear concentration-dependent inhibition of the recognition of collagen XIα1 by the RRHTEGMQA peptide, but not by the EGMQADADD peptide or by the KRTISIWGT scrambled control peptide.

These results show that both the anti-colXIα1 clone 3 and anti-colXIα1 clone 9 supernatants specifically recognize the RRHTEGMQA sequence of the human collagen XIα1 putative C-telopeptide. The lack of inhibition of this recognition by the EGMQADADD peptide points to a prominent role of the RRHT amino acid residues in the recognition of the epitope by these two mAbs.

As genetic characterization of these two clones demonstrated that they were almost identical (see below), the following observations were made only for the anti-colXIα1 clone 9 mAb.

The recombinant COL11A1 Fusion Protein from Proteintech was also assayed in an ELISA and Western blot with finally purified preparations of the anti-colXIα1 clone 9 and PLY-7 mAbs. Table 4 shows their ELISA immunoreactivity characteristics. The anti-colXIα1 clone 9 mAb was reactive with collagen XIα1 and, to a lesser extent, with the COL11A1 Fusion Protein, but not with the C-propeptide, which lacks the RRHTEGMQA sequence and served as a negative control.

Table 4. The ELISA immunoreactivity of the finally purified preparations of the anti-colXIα1 clone 9 and PLY-7 mAbs.

	ELISA [a] Antigen (1 µg/100 µL Well)		
Purified mAb (1 µg/100 µL Well)	**Recombinant COL11A1 Fusion Protein (Proteintech)**	**Recombinant Collagen XIα1 (GenScript)**	**Recombinant C-Propeptide (GenScript)**
Anti-colXIα1 clone 9	0.699	2.228	0.012
Irrelevant PLY-7	0.250	0.116	0.109

[a] ELISA optical density scale of blanked data from 0 to 4.

In the Western blots, the anti-olXIa1 clone 9 mAb recognized recombinant collagen XIa1 protein (containing degradation products), as well as the COL11A1 Fusion Protein, but not the C-propeptide, from 12% SDS-PAGE gels and 15 µg/lane of purified proteins, as shown in Figure 5 (see also Supplementary Material S1).

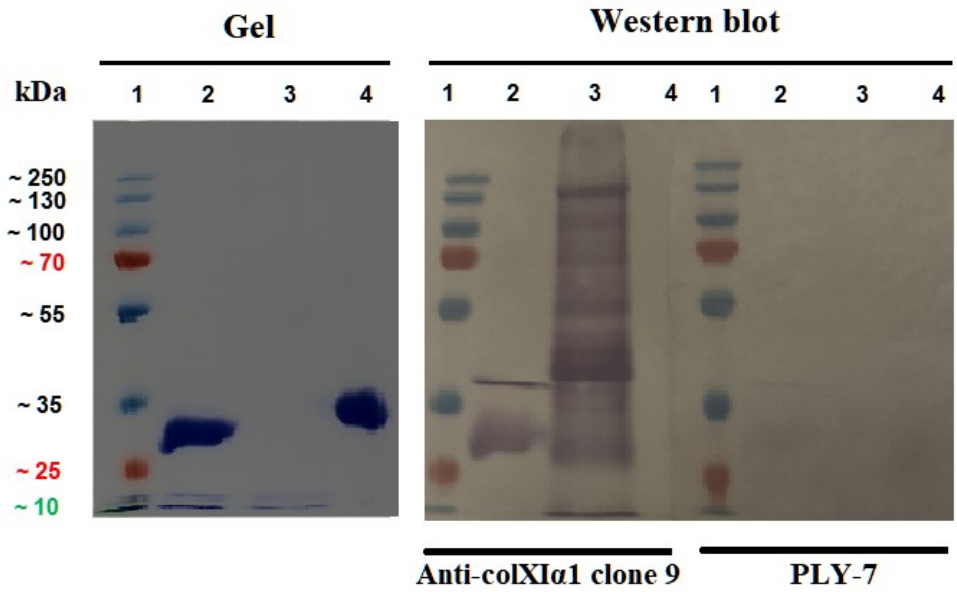


Figure 5. SDS-PAGE gel staining and Western blot of recombinant antigens with finally purified preparations of anti-colXIα1 clone 9 and PLY-7 mAbs. Lane 1: PageRuler™ Plus Prestained Protein Ladder. Lane 2: COL11A1 Fusion Protein (Proteintech). Lane 3: Collagen XIα1 (GenScript). Lane 4: C-propeptide (GenScript). Western blot color development was monitored following the manufacturer's instructions. Full-length blots/gels are presented in Supplementary Figure S1.

These last results confirm that the anti-colXIα1 clone 9 mAb specifically recognizes the RRHTEGMQA amino acid stretch, which is contained in the sequence of the putative C-telopeptide of human collagen XIα1, according to both the ELISA and Western blot.

3.2. Peptide Structure Predictions

The structures of peptide sequences corresponding to some of the assayed antigens were predicted using the PEP-FOLD4 server to gain more insight into the nature of the epitope recognized by the anti-colXIα1 clone 9 mAb.

Figure 6A shows the structural prediction of the 50 N-terminal amino acid sequence PLPILSSKKTRRHTEGMQADADDNILDYSDGMEEIFGSLNSLKQDIEHMK of the COL11A1 Fusion Protein from Proteintech, with the first 19 N-terminal PLPILSSKKTRRHTEGMQA amino acid residues of the putative C-telopeptide. In this model, the RRH

amino acid residues are part of a disordered stretch preceding an α-helix. The prediction for the free RRHTEGMQA peptide is a frank α-helix (panel B). The structural prediction (panel C) for the C-terminal RRHTEGMQA stretch is very similar to the one in panel A for the last-50 amino acid sequence NKGSTGPAGQKGDSGLPGPPGSPGPPGEVIQ-PLPILSSKKTRRHTEGMQA of GenScript's recombinant collagen XIα1 form, whose last 21-C-terminal IQPLPILSSKKTRRHTEGMQA amino acid residues correspond to the putative C-telopeptide. In these in silico-generated models, the RRHTEGMQA amino acid stretch adopts a conformation consistent with a linear epitope accessible to antibodies. Thus, according to these predictions and the ELISA and Western blot results, the epitope recognized by the anti-colXIα1 clone 9 mAb behaves like a linear epitope, with essential recognition of the RRHT amino acid residues.

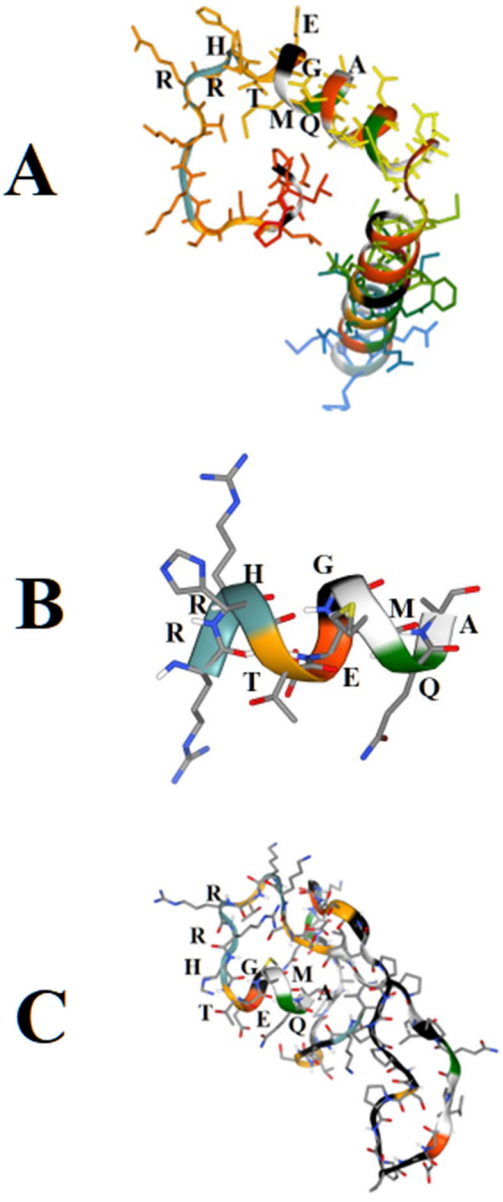


Figure 6. The PEP-FOLD4-derived structural predictions of peptides related to the putative C-telopeptide. (**A**): The 50 N-terminal amino acid sequence of the COL11A1 Fusion Protein from Proteintech, with the first 19 N-terminal PLPILSSKKTRRHTEGMQA amino acid residues of the putative C-telopeptide. (**B**): A free RRHTEGMQA peptide. (**C**): The 50 C-terminal amino acid sequence of GenScript's recombinant collagen XIα1 form, whose last 21 C-terminal IQPLPILSSKKTRRHTEGMQA amino acid residues correspond to the putative C-telopeptide. The peptide's N-terminus is on the left in (**A**,**B**) and on the right in (**C**).

3.3. Genetic Characterization of the Two mAbs

Sequencing of the whole heavy and light chains of the two mAbs confirmed that both were IgG1, kappa.

Table 5 shows the most probable usage of germinal V(D)J gene segments by the two mAbs, according to the IMGT/V-QUEST (https://www.imgt.org/IMGTindex/V-QUEST.php) analysis (accessed on 9 January 2024). The V_H and V_L domains of the two mAbs were most likely formed from the same germinal gene segments.

Table 5. The most probable germinal V(D)J gene segment usage by the two mAbs.

mAb	V_H	D_H	J_H	V_{kappa}	J_{kappa}
Anti-colXIα1 clone 3	IGHV9-2-1*01 F	IGHD2-10*01 F	IGHJ3*01 F	IGKV8-30*01 F	IGKJ4*01 F
Anti-colXIα1 clone 9	IGHV9-2-1*01 F	IGHD2-10*01 F	IGHJ3*01 F	IGKV8-30*01 F	IGKJ4*01 F

Table 6 shows the amino acid sequences of the CDRs of the two mAbs, as identified using IMGT/V-QUEST. With very limited nucleotide differences, the CDRs of the V_H domains of the two mAbs are almost identical, as well as the CDRs of their V_L domains, which explains their very similar monoclonal reactivity.

Table 6. Deduced IMGT CDRs.

mAb	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
Anti-colXIα1 clone 3	GYTFTDYS	INTETG**E**P	**V**RRANYGNAWF**V**Y	Q**S**LLYRSN**Q**KNY	WAS	QQYYDYPFT
Anti-colXIα1 clone 9	GYTFTDYS	INTETG**Q**P	**I**RRANYGNAWF**A**Y	Q**N**LLYRSN**H**KNY	WAS	QQYYDYPFT

Amino acids that are not identical between the mAbs are in bold.

When the sequences of these CDRs were compared with sequence databases, some individual CDRs were found to be present in different immunoglobulins, but neither the combination of the three CDRs of each V domain nor the combination of the six CDRs of each of the two mAbs was identified in any immunoglobulin reported so far. This result reinforces the novelty of these mAbs.

For patent purposes, per The Budapest Treaty of 1977, the anti-colXIα1 clone 3 and anti-colXIα1 clone 9 mouse hybridomas were deposited in the European Collection of Authenticated Cell Cultures (ECACC) repository with accession numbers 23112901 and 23112902, respectively, given by the International Depositary Authority. They were determined to be free of mycoplasma contamination.

The V_H and V_L nucleotide sequences of the anti-colXIα1 clone 3 and anti-colXIα1 clone 9 mAbs were deposited in the GenBank Nucleotide Sequence Database [21] with accession numbers PP150425 and PP150426, and PP150423 and PP150424, respectively.

3.4. Immunocytochemistry (ICC) and Immunohistochemistry (IHC) Analyses

Immunocytochemistry and immunohistochemistry analyses were performed with the finally purified preparations of the anti-colXIα1 clone 9 and PLY-7 mAbs.

As shown in Figure 7, the anti-colXIα1 clone 9 mAb developed a rather faint immunostaining in the well-known *COL11A1*-positive A204 and NCI-H661 cell lines, but not in the *COL11A1*-quasi-negative A549 cell line. These three cell lines are known to express the cytoskeletal intermediate filament vimentin; as such, their anti-vimentin staining served as an ICC positive control.

No clear immunostaining patterns were observed in any of the IHC pancreatic ductal adenocarcinoma (PDAC) specimens studied.

Therefore, under the current conditions, the anti-colXIα1 clone 9 mAb did not provide robust detection of collagen XIα1 in either cultured cells or tissue sections.

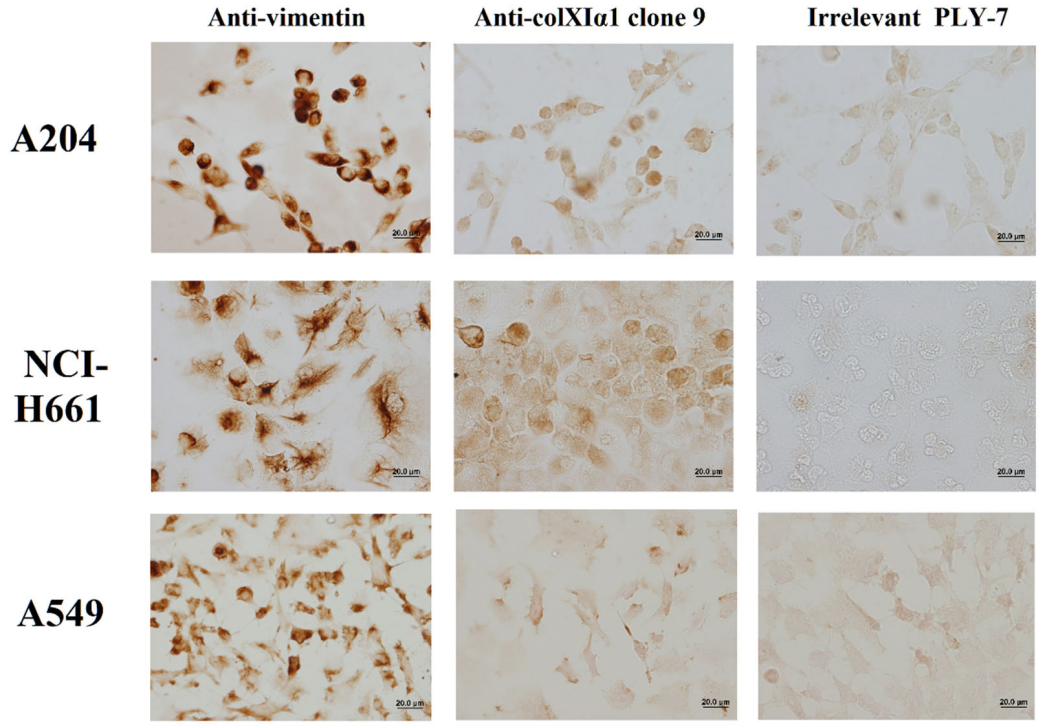


Figure 7. Representative images of immunostaining of cultured cancer cell lines with the purified anti-colXIα1 clone 9 and irrelevant PLY-7 mAbs. Original magnification: 400×; scale bar: 20.0 µm.

4. Discussion

At present, the molecular biology underlying the expression of human procollagen XIα1 is far from being understood. In particular, the function and structural characteristics of (pro)collagen XIα1 have been studied in tendons, vitreous humor, and cartilage from mice, rats, cows, and chickens, among other origins and body locations [22,23], but not in humans. No detailed molecular studies have been conducted on human cancer either. A purified recombinant form of human procollagen XIα1 is not available, and no study has been carried out on its C-terminal enzymatic processing with furin or BMP-1, as in the case of human procollagen Vα1 [24].

Based on its similarity to other collagens, it is assumed that the terminal C-telopeptide of the mature human collagen XIα1 corresponds to the (1543) IQPLPILSSKK-TRRHTEGMQA (1563) amino acid sequence of procollagen XIα1. According to diverse studies, the C-telopeptide would be involved in intrafibrillar cross-linking with the triple-helical regions of adjacent molecules. Minor collagen XIα1 would act as a nucleator in the assembly of extracellular collagen fibrils [14,22,23]. Moreover, being massively surrounded by major I, II, and III fibrillar collagens [14], this C-telopeptide could remain buried and not exposed on the surface of fibrils, thus hindering its recognition by antibodies. Nevertheless, it could be exposed at the time of enzymatic cleavage by extracellular proteinases and during further processing steps.

In the case of human collagens Iα1, IIα1, and IIIα1, soluble C-terminal cross-linked telopeptides (CTXs) are released and may be detected in biological fluids for diagnostic purposes [25–28], thereby serving as biomarkers of bone resorption or osteoarthritis. Different antibody preparations, which are used in immunoassays, are available for detecting

and measuring these CTXs and for the immunostaining of extracellular collagens [29,30]. However, this is not the case for human collagen XIα1 at present.

This study aimed to rescue and partially characterize two mAbs—anti-colXIα1 clone 3 and anti-colXIα1 clone 9—targeting the putative C-telopeptide amino acid stretch of the mature human collagen XIα1. They recognize the RRHTEGMQA amino acid sequence but not the EGMQADADD sequence, which includes the DADD amino acids of the N-terminus of the C-propeptide, or the recombinant C-propeptide form. In humans, this RRHTEGMQA nine-amino-acid sequence is only present as such in (pro)collagen XIα1, and is partially found in some other unrelated proteins.

These two mAbs used the same set of V, D, and J gene segments for the construction of their V domains, and their CDRs are almost identical. Their reactivity is also very similar.

Based on structural predictions (which, with the limitations and caveats associated, should be taken as hypothetical models) and the ELISA and Western blot assay results, the anti-colXIα1 clone 9 mAb recognizes a linear epitope within the C-telopeptide, in which the RRHT amino acid residues are central to mAb recognition.

Altogether, recalling the low-intensity immunostaining of cultured cells by the anti-colXIα1 clone 9 mAb, compared with its reactivity with the free collagen XIα1 recombinant form and aligning with the putative C-terminal processing of procollagen XIα1, the RRHTEGMQA epitope sequence should be primarily envisaged as a linear B-cell neoepitope, as it is not identified as such in procollagen.

A plethora of antibodies that are reactive with respect to diverse collagens has been reported and described. A few of these recognize B-cell neoepitopes, which become exposed after enzymatic processing [31–36]. Thus, the epitope recognized by the anti-colXIα1 clone 9 mAb may be added to the list of collagen B-cell neoepitopes.

The purified anti-colXIα1 clone 9 mAb did not stain fibroblast-like cells associated with carcinoma cell niches on tissue sections from human PDAC samples, as the 1E8.33 mAb does [37]; additionally, no staining of extracellular matrix components was observed in A204 cell cultures. These observations seem to corroborate the suggested buried status of the C-telopeptide of human collagen XIα1 in the extracellular matrix of the tumor microenvironment. Alternatively, the conformation adopted by the RRHTEGMQA stretch, or any derivatization so far not identified in vivo, would make this C-telopeptide not recognized by this mAb.

A human collagen Iα2 C-telopeptide-binding antibody has been shown to reduce the rate of cleavage of the C-terminal propeptide by BMP-1 and to limit extracellular fibril formation both in vitro and in organotypic keloid-like constructs [31,38]. Blocking antibodies to secretory components of myofibroblastic CAFs have been shown to affect carcinoma progression; for instance, an antibody to the microfibrillar-associated protein 5 (MFAP5) suppressed tumor growth in vivo, with a concurrent reduction in the expression of the *COL11A1* gene [39]. Similarly, an antibody to MMP-11 showed significant anti-tumoral effects in animal models [40], as did a humanized antibody to secretory collagen triple-helix repeat-containing-1 (CTHRC1) protein [41]. Therefore, antibodies to the C-telopeptide of the mature human collagen XIα1 could show a progression-restraining capacity of tumors expressing the *COL11A1* gene by interfering with (pro)collagen processing steps, thus also pointing to therapeutic potential.

One main limitation of this study is that there was no purified recombinant form of human procollagen XIα1, and it was not assayed for its experimental enzymatic processing. Therefore, the actual nature and characteristics of the putative C-telopeptide of human collagen XIα1, and whether our mAbs recognize the product(s) resulting from in vitro and in vivo processing, remain to be precisely determined.

5. Conclusions and Future Perspectives

In this study, highly specific mAbs to the putative C-telopeptide of human collagen XIα1 were generated for the first time. They seem to recognize a unique linear B-cell neoepitope, while the "neoepitope" concept remains to be confirmed through native procollagen processing experiments. These mAbs may be helpful to researchers analyzing the cell biology of human (pro)collagen XIα1 and for better characterization of *COL11A1*-positive tumors. Furthermore, if they can interfere with *COL11A1*-expressing stromal and cancer cells, they could also have in vivo therapeutic potential.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/antib15020021/s1: Supplementary Material S1: 12% SDS-PAGE gel staining and Western blot of recombinant antigens with finally purified preparations of anti-colXIα1 clone 9 and PLY-7 mAbs. Lane 1: C-propeptide (GenScript). Lane 2: COL11A1 Fusion Protein (Proteintech). Lane 3: PageRuler™ Plus Prestained Protein Ladder. Western blot color development was monitored following the manufacturer´s instructions; Supplementary Figure S1: the original pictures taken from gel and Western blot analyses of Figure 5.

Author Contributions: Conceptualization; J.R.d.l.T. and L.J.G.-F.; data curation: J.R.d.l.T.; formal analysis: M.G.-O., L.L.-O., S.R.-R., J.R.d.l.T. and I.F.-V.; funding acquisition: M.L.-M. and L.J.G.-F.; investigation: M.G.-O., L.L.-O., S.R.-R., J.R.d.l.T. and P.R.-F.; methodology; M.G.-O., L.L.-O., S.R.-R., J.R.d.l.T. and I.F.-V.; project administration: L.J.G.-F.; resources: M.G.-O., I.F.-V. and L.J.G.-F.; software; J.R.d.l.T.; supervision: J.R.d.l.T., M.L.-M. and L.J.G.-F.; validation: M.G.-O., L.L.-O., S.R.-R., J.R.d.l.T. and I.F.-V.; visualization: M.G.-O., L.L.-O., S.R.-R., P.R.-F., I.F.-V., M.L.-M., J.R.d.l.T. and L.J.G.-F.; writing—original draft preparation: J.R.d.l.T.; writing—review and editing: J.R.d.l.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially financed by Startquake, S.L. (ETERNA Diagnostics), Spain, and Proyecto IDI/2021/000282, FICYT, the Principality of Asturias, Spain, provided to Luis J. García-Flórez.

Institutional Review Board Statement: This study was conducted per the Declaration of Helsinki and approved by the Ethical and Scientific Committees of the Principality of Asturias (project no. 42/12; reference: CEImPA 2022.462 (6 February 2023)). The human specimens obtained after giving informed consent to participate were from the Principality of Asturias BioBank (PT23/077). Mice were handled following the Guidelines of the Experimental Animal Ethics Committee of the University of Oviedo and after the approval of animal experimental procedures by the Government of the Principality of Asturias, Spain, Resolutions *PROAE 62/2019* (19 December 2019) and *PROAE 4/2022* (21 March 2022).

Data Availability Statement: The V_H and V_L nucleotide sequences of the anti-colXIα1 clone 3 and anti-colXIα1 clone 9 mAbs were deposited in the GenBank Nucleotide Sequence Database [21] with accession numbers PP150425 and PP150426 and PP150423 and PP150424, respectively. Anti-colXIα1 clone 9 and PLY-7 mAbs may be sent upon request, depending on the available stocks.

Acknowledgments: The authors greatly appreciate the excellent technical assistance of María Álvarez-García, Ana M. López-Fernández, and Lucía Ronderos-López (the Molecular Histopathology Unit in Animal Models of Cancer, IUOPA); Marta M. Alonso-Guervós (the Photonic Microscopy and Image Processing Unit of the Scientific and Technical Services [SCTs] of the University of Oviedo); and M. Daniela Corte-Torres (Biobank of the Principality of Asturias; ISPA-FINBA). We want to particularly acknowledge the patients and the Biobank of the Principality of Asturias (BioPA) (National Registry of Biobanks B.0000827) (PT23/00077 funded by ISCIII and co-funded by the European Union), integrated into the Platform ISCIII Biobanks and Biomodels, for their collaboration.

Conflicts of Interest: Marcos Ladreda-Mochales is a shareholder of Startquake, S.L.

Abbreviations

The following abbreviations were used in this manuscript:

ADCs	Antibody–Drug Conjugates
ATCC	American Type Culture Collection
BMP-1	Bone Morphogenetic Protein-1
BSA	Bovine Serum Albumin
CAFs	Cancer-Associated Fibroblasts
CDRs	Complementarity-Determining Regions
CHO cells	Chinese Hamster Ovary Cells
COL11A1	Collagen Type XI Alpha 1 Chain Gene
ColXIα1	Collagen XIα1
CTHRC1	Collagen Triple-Helix Repeat-Containing-1
CTXs	Cross-Linked Telopeptides
DAB	Diaminobenzidine
ELISA	Enzyme-Linked Immunosorbent Assay
ECACC	European Collection of Authenticated Cell Cultures
FBS	Fetal Bovine Serum
GST	Glutathione S-Transferase
HAT	Hypoxanthine–Aminopterin–Thymidine
HRPO	Horseradish Peroxidase
ICC	Immunocytochemistry
IHC	Immunohistochemistry
INHBA	Inhibin Subunit Beta A gene
ITGA11	Integrin Alpha-11
KLH	Keyhole Limpet Hemocyanin
LRRC15	Leucine-Rich Repeat-Containing 15
mAbs	Mouse Monoclonal Antibodies
MFAP5	Microfibrillar-Associated Protein 5
MMP-11	Matrix Metalloproteinase-11
PDAC	Pancreatic Ductal Adenocarcinoma
PBS	Phosphate-Buffered Saline
SA	Sodium Azide
SDS-PAGE	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
TGF-β1	Transforming Growth Factor Beta1
THBS2	Thrombospondin 2 gene
TMB	Tetramethylbenzidine

References

1. Kim, H.; Watkinson, J.; Varadan, V.; Anastassiou, D. Multi-cancer computational analysis reveals invasion-associated variant of desmoplastic reaction involving INHBA, THBS2 and COL11A1. *BMC Med. Genom.* **2010**, *3*, 51. [CrossRef] [PubMed]
2. Raglow, Z.; Thomas, S.M. Tumor matrix protein collagen XIα1 in cancer. *Cancer Lett.* **2015**, *357*, 448–453. [CrossRef] [PubMed]
3. Vázquez-Villa, F.; García-Ocaña, M.; Galván, J.A.; García-Martínez, J.; García-Pravia, C.; Menéndez-Rodríguez, P.; González-del Rey, C.; Barneo-Serra, L.; de Los Toyos, J.R. COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human invasive carcinoma-associated stromal cells and carcinoma progression. *Tumour Biol.* **2015**, *36*, 2213–2222. [CrossRef] [PubMed]
4. Nallanthighal, S.; Heiserman, J.P.; Cheon, D.J. Collagen Type XI Alpha 1 (COL11A1): A Novel Biomarker and a Key Player in Cancer. *Cancers* **2021**, *13*, 935. [CrossRef] [PubMed]
5. Popova, N.V.; Jücker, M. The Functional Role of Extracellular Matrix Proteins in Cancer. *Cancers* **2022**, *14*, 238. [CrossRef]
6. Freire, J.; García-Berbel, P.; Caramelo, B.; García-Berbel, L.; Ovejero, V.J.; Cadenas, N.; Azueta, A.; Gómez-Román, J. Usefulness of COL11A1 as a Prognostic Marker of Tumor Infiltration. *Biomedicines* **2023**, *11*, 2496. [CrossRef] [PubMed]
7. Dominguez, C.X.; Müller, S.; Keerthivasan, S.; Koeppen, H.; Hung, J.; Gierke, S.; Breart, B.; Foreman, O.; Bainbridge, T.W.; Castiglioni, A.; et al. Single-Cell RNA Sequencing Reveals Stromal Evolution into LRRC15^{+} Myofibroblasts as a Determinant of Patient Response to Cancer Immunotherapy. *Cancer Discov.* **2020**, *10*, 232–253. [CrossRef] [PubMed]

8. Zhu, K.; Cai, L.; Cui, C.; de Los Toyos, J.R.; Anastassiou, D. Single-cell analysis reveals the pan-cancer invasiveness-associated transition of adipose-derived stromal cells into COL11A1-expressing cancer-associated fibroblasts. *PLoS Comput. Biol.* **2021**, *17*, e1009228. [CrossRef] [PubMed]
9. Zhang, J.; Lu, S.; Lu, T.; Han, D.; Zhang, K.; Gan, L.; Wu, X.; Li, Y.; Zhao, X.; Li, Z.; et al. Single-cell analysis reveals the COL11A1^{+} fibroblasts are cancer-specific fibroblasts that promote tumor progression. *Front. Pharmacol.* **2023**, *14*, 1121586. [CrossRef] [PubMed]
10. Thorlacius-Ussing, J.; Jensen, C.; Nissen, N.I.; Cox, T.R.; Kalluri, R.; Karsdal, M.; Willumsen, N. The collagen landscape in cancer: Profiling collagens in tumors and in circulation reveals novel markers of cancer-associated fibroblast subtypes. *J. Pathol.* **2024**, *262*, 22–36. [CrossRef] [PubMed]
11. Nissen, N.I.; Karsdal, M.A.; Cox, T.R.; Willumsen, N. Chapter 38—The roles of collagens and fibroblasts in cancer. In *Biochemistry of Collagens, Laminins and Elastin*, 3rd ed.; Morten, A.K., Ed.; Academic Press, Elsevier B.V.: Amsterdam, The Netherlands, 2024; pp. 419–434, ISBN 9780443156175. [CrossRef]
12. The Human Protein Atlas. Available online: https://www.proteinatlas.org/ENSG00000060718-COL11A1/cell+line (accessed on 21 January 2026).
13. UniProt. Available online: https://www.uniprot.org/uniprotkb/P12107/entry (accessed on 21 January 2026).
14. Bella, J.; Hulmes, D.J.S. Fibrillar Collagens. *Subcell. Biochem.* **2017**, *82*, 457–490. [CrossRef] [PubMed]
15. Nissen, N.I.; Kehlet, S.; Johansen, A.Z.; Chen, I.M.; Karsdal, M.; Johansen, J.S.; Diab, H.M.H.; Jørgensen, L.N.; Sun, S.; Manon-Jensen, T.; et al. Noninvasive prognostic biomarker potential of quantifying the propeptides of Type XI collagen alpha-1 chain (PRO-C11) in patients with pancreatic ductal adenocarcinoma. *Int. J. Cancer* **2021**, *149*, 228–238. [CrossRef] [PubMed]
16. Liddell, J.R.; Cryer, A. *A Practical Guide to Monoclonal Antibodies*; John Wiley and Sons Ltd.: Chichester, UK, 1991. Available online: https://www.wiley.com/en-us/A+Practical+Guide+to+Monoclonal+Antibodies-p-9780471929055 (accessed on 21 January 2026).
17. de los Toyos, J.R.; Méndez, F.J.; Aparicio, J.F.; Vázquez, F.; Del Mar García Suárez, M.; Fleites, A.; Hardisson, C.; Morgan, P.J.; Andrew, P.W.; Mitchell, T.J. Functional analysis of pneumolysin by use of monoclonal antibodies. *Infect. Immun.* **1996**, *64*, 480–484. [CrossRef] [PubMed]
18. Suárez-Alvarez, B.; García-Suárez, M.d.M.; Méndez, F.J.; de los Toyos, J.R. Characterisation of mouse monoclonal antibodies for pneumolysin: Fine epitope mapping and V gene usage. *Immunol. Lett.* **2003**, *88*, 227–239. [CrossRef] [PubMed]
19. Rey, J.; Murail, S.; de Vries, S.; Derreumaux, P.; Tufféry, P. PEP-FOLD4: A pH-dependent force field for peptide structure prediction in aqueous solution. *Nucleic Acids Res.* **2023**, *51*, W432–W437.
20. Manso, T.; Folch, G.; Giudicelli, V.; Jabado-Michaloud, J.; Kushwaha, A.; Nguefack Ngoune, V.; Georga, M.; Papadaki, A.; Debbagh, C.; Pégorier, P.; et al. IMGT® databases, related tools and web resources through three main axes of research and development. *Nucleic Acids Res.* **2022**, *50*, D1262–D1272. [CrossRef] [PubMed]
21. Benson, D.A.; Cavanaugh, M.; Clark, K.; Karsch-Mizrachi, I.; Lipman, D.J.; Ostell, J.; Sayers, E.W. GenBank. *Nucleic Acids Res.* **2013**, *41*, D36–D42. [CrossRef] [PubMed]
22. Sun, M.; Luo, E.Y.; Adams, S.M.; Adams, T.; Ye, Y.; Shetye, S.S.; Soslowsky, L.J.; Bir, D.E. Collagen XI regulates the acquisition of collagen fibril structure, organization and functional properties in tendon. *Matrix Biol.* **2020**, *94*, 77–94. [CrossRef] [PubMed]
23. Luo, Y.Y.; Szlarski, P.M.; Kehlet, S.N.; Karsdal, M.A. Chapter 11—Type XI collagen. In *Biochemistry of Collagens, Laminins and Elastin*, 2nd ed.; Morten, A.K., Ed.; Academic Press, Elsevier B.V.: Amsterdam, The Netherlands, 2019; pp. 99–106, ISBN 978-0-12-817068-7. [CrossRef]
24. Kessler, E.; Fichard, A.; Chanut-Delalande, H.; Brusel, M.; Ruggiero, F. Bone morphogenetic protein-1 (BMP-1) mediates C-terminal processing of procollagen V homotrimer. *J. Biol. Chem.* **2001**, *276*, 27051–27057. [CrossRef]
25. Fledelius, C.; Johnsen, A.H.; Cloos, P.A.; Bonde, M.; Qvist, P. Characterization of urinary degradation products derived from type I collagen. Identification of a beta-isomerized Asp-Gly sequence within the C-terminal telopeptide (alpha1) region. *J. Biol. Chem.* **1997**, *272*, 9755–9763. [CrossRef] [PubMed]
26. Chubb, S.A. Measurement of C-terminal telopeptide of type I collagen (CTX) in serum. *Clin. Biochem.* **2012**, *45*, 928–935. [CrossRef] [PubMed]
27. Huang, M.; Zhao, J.; Huang, Y.; Dai, L.; Zhang, X. Meta-analysis of urinary C-terminal telopeptide of type II collagen as a biomarker in osteoarthritis diagnosis. *J. Orthop. Translat.* **2017**, *13*, 50–57. [CrossRef] [PubMed]
28. Necula, L.; Matei, L.; Dragu, D.; Pitica, I.; Neagu, A.; Bleotu, C.; Diaconu, C.C.; Chivu-Economescu, M. Collagen Family as Promising Biomarkers and Therapeutic Targets in Cancer. *Int. J. Mol. Sci.* **2022**, *23*, 12415. [CrossRef] [PubMed]
29. Srivastava, A.K.; MacFarlane, G.; Srivastava, V.P.; Mohan, S.; Baylink, D.J. A new monoclonal antibody ELISA for detection and characterization of C-telopeptide fragments of type I collagen in urine. *Calcif. Tissue Int.* **2001**, *69*, 327–336. [CrossRef] [PubMed]
30. Anti-Collagen 1, Alpha 1 Telopeptide Antibody SKU: 322-COLT. Available online: https://www.aveslabs.com/products/anti-collagen-1-alpha-1-telopeptide-antibody-322-colt (accessed on 20 January 2026).
31. Chung, H.J.; Steplewski, A.; Chung, K.Y.; Uitto, J.; Fertala, A. Collagen fibril formation. A new target to limit fibrosis. *J. Biol. Chem.* **2008**, *283*, 25879–25886. [CrossRef] [PubMed]

32. Barascuk, N.; Veidal, S.S.; Larsen, L.; Larsen, D.V.; Larsen, M.R.; Wang, J.; Zheng, Q.; Xing, R.; Cao, Y.; Rasmussen, L.M.; et al. A novel assay for extracellular matrix remodeling associated with liver fibrosis: An enzyme-linked immunosorbent assay (ELISA) for a MMP-9 proteolytically revealed neo-epitope of type III collagen. *Clin. Biochem.* **2010**, *43*, 899–904. [CrossRef] [PubMed]
33. Leeming, D.J.; He, Y.; Veidal, S.; Nguyen, Q.; Larsen, D.; Koizumi, M.; Segovia-Silvestre, T.; Zhang, C.; Zheng, Q.; Sun, S.; et al. A novel marker for assessment of liver matrix remodeling: An enzyme-linked immunosorbent assay (ELISA) detecting a MMP generated type I collagen neo-epitope (C1M). *Biomarkers* **2011**, *16*, 616–628. [CrossRef] [PubMed]
34. Veidal, S.S.; Karsdal, M.A.; Nawrocki, A.; Larsen, M.R.; Dai, Y.; Zheng, Q.; Hägglund, P.; Vainer, B.; Skjøt-Arkil, H.; Leeming, D.J. Assessment of proteolytic degradation of the basement membrane: A fragment of type IV collagen as a biochemical marker for liver fibrosis. *Fibrogenesis Tissue Repair* **2011**, *4*, 22. [CrossRef]
35. Willumsen, N.; Bager, C.L.; Leeming, D.J.; Smith, V.; Karsdal, M.A.; Dornan, D.; Bay-Jensen, A.-C. Extracellular matrix specific protein fingerprints measured in serum can separate pancreatic cancer patients from healthy controls. *BMC Cancer* **2013**, *13*, 554. [CrossRef]
36. Brachmann, C.B.; Zhang, Y.; Zavodovskaya, M.; Hu, J.; Maltzman, J.D.; Smith, V.; Xiao, Y.; Patterson, S.D. Evaluating collagen neoepitopes as pharmacodynamic biomarkers of GS-5745, an MMP9 inhibitor, in advanced gastric cancer. *J. Clin. Oncol.* **2017**, *35*, 58. [CrossRef]
37. García-Ocaña, M.; Vázquez, F.; García-Pravia, C.; Fuentes-Martínez, N.; Menéndez-Rodríguez, P.; Fresno-Forcelledo, F.; Barneo-Serra, L.; Del Amo-Iribarren, J.; Simón-Buela, L.; De Los Toyos, J.R. Characterization of a novel mouse monoclonal antibody, clone 1E8.33, highly specific for human procollagen 11A1, a tumor-associated stromal component. *Int. J. Oncol.* **2012**, *40*, 1447–1454. [CrossRef]
38. Steplewski, A.; Fertala, A. Inhibition of collagen fibril formation. *Fibrogenesis Tissue Repair* **2012**, *5*, S29. [CrossRef] [PubMed]
39. Yeung, T.L.; Leung, C.S.; Yip, K.; Sheng, J.; Vien, L.; Bover, L.C.; Birrer, M.J.; Wong, S.T.C.; Mok, S.C. Anticancer Immunotherapy by MFAP5 Blockade Inhibits Fibrosis and Enhances Chemosensitivity in Ovarian and Pancreatic Cancer. *Clin. Cancer Res.* **2019**, *25*, 6417–6428. [CrossRef] [PubMed]
40. Yang, H.; Jiang, P.; Liu, D.; Wang, H.Q.; Deng, Q.; Niu, X.; Lu, L.; Dai, H.; Wang, H.; Yang, W. Matrix Metalloproteinase 11 Is a Potential Therapeutic Target in Lung Adenocarcinoma. *Mol. Ther. Oncolytics* **2019**, *14*, 82–93. [CrossRef] [PubMed]
41. Kang, M.K.; Jiang, F.; Kim, Y.J.; Ryu, K.; Masamune, A.; Hamada, S.; Park, Y.Y.; Koh, S.S. CTHRC1 Induces Pancreatic Stellate Cells (PSCs) into Myofibroblast-like Cancer-Associated Fibroblasts (myCAFs). *Cancers* **2023**, *15*, 3370. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.