Therapeutic Targeting of Tumor Collagen

Subjects: Oncology | Immunology | Medicine, Research & Experimental

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The tumor stroma, which comprises stromal cells and non-cellular elements, is a critical component of the tumor microenvironment (TME). The dynamic interactions between the tumor cells and the stroma may promote tumor progression and metastasis and dictate resistance to established cancer therapies. Therefore, novel antitumor approaches should combine anticancer and anti-stroma strategies targeting dysregulated tumor extracellular matrix (ECM). ECM remodeling is a hallmark of solid tumors, leading to extensive biochemical and biomechanical changes, affecting cell signaling and tumor tissue three-dimensional architecture. Increased deposition of fibrillar collagen is the most distinctive alteration of the tumor ECM. Consequently, several anticancer therapeutic strategies have been developed to reduce excessive tumor collagen deposition.

Keywords: collagen ; tumor microenvironment ; extracellular matrix ; cancer-associated fibroblast ; tumor-stroma interaction ; desmoplasia ; stomal cells

1. Introduction

Tumor and tumor-associated stromal cells promote the production and remodeling of the extracellular matrix (ECM) to create a tumor microenvironment (TME) that supports cancer growth, metastatic dissemination, and immune evasion and affects the patient's response to therapy [1]. ECM is composed of a vast array of proteins, proteoglycans and glycosaminoglycans organized in a complex and dynamic three-dimensional network. The members of the collagen family are the most abundant (up to 90%) proteins in the ECM [2]. Collagen synthesis and assembly are a complex, multistep process involving different specific enzymes and molecular chaperones that are tightly regulated to preserve tissue homeostasis. Collagens are composed of three homo- or hetero-trimeric polypeptide chains (α chains), which are synthesized as pre-pro-collagens that undergo several post-translational modifications, including proline and lysine hydroxylation and glycosylation, in the endoplasmic reticulum. Three post-translationally modified pro-α chains form a procollagen molecule, which, upon secretion into the extracellular space, is proteolytically cleaved. Triple-helical procollagen is transported across the Golgi complex, self-assembled into collagen fibrils and exported into the ECM. The fibrils are then stabilized by the formation of covalent crosslinks and aggregation of multiple collagen fibrils to finally produce collagen fibers [3]. Among the 28 isoforms of collagen identified in humans, the types I, II, III, V, XI, XXIV and XXVII constitute the sub-group of fibrillar collagen, which organizes a three-dimensional framework that supports the ECM's mechanical strength and regulates cell adhesion, migration, differentiation, and survival [4]. Each collagen isoform has a distinct tissue distribution and might exert diverse functions in cancer-associated processes [5]. In particular, fibrillar collagen types I and III are the most abundant isoforms of collagen and have been associated with different types of tumors, including bone, breast, colorectal, ovarian, lung, head and neck, and pancreatic cancers [6].

In cancer, the ECM structure, physical properties, metabolism, and function are highly dysregulated. In particular, the tumor ECM is more abundant, condensed, and stiffer than the ECM in the surrounding healthy tissue, leading to increased interstitial fluid pressure and making the tumor less accessible to nutrients, oxygen, immune cells, and therapeutic drugs [7]. Above all, collagens are upregulated in several types of cancer, such as oral squamous cell carcinoma, breast, pancreatic, and gastric cancers; moreover, high collagen expression correlates with poor overall survival and affects the response to chemo-, radio- and immuno-therapies [8][9]. Collagen has a prognostic and predictive value in different types of solid tumors, including breast, prostate, lung, liver, colon, and pancreatic cancers [10][11]. In particular, oriented collagen around tumor cells [12] and the identification of distinct collagen organization patterns, termed tumor-associated collagen signatures (TACS), are indicators of disease progression [13]. Recently, the predictive value of collagen has been extended from tissue to blood, as non-invasive determination of serum collagen fragments has been proposed for the optimization of patient selection to improve the efficacy of immune checkpoint inhibitor (ICI) immunotherapy [14].

Cancer cell behavior is modulated via a biochemical and biomechanical cross-talk with stromal cells, mainly cancer-associated fibroblasts (CAFs) [15][16]. Different subsets of CAFs have been identified on the basis of their gene expression, phenotypic marker profiles and functions [17][18]. Among these sub-types, the myofibroblast-like CAFs (myCAFs) express high levels of fibroblast activation protein (FAP), secrete cytokines, chemokines, and extracellular vesicles and produce a dense collagen-rich ECM that modulates the infiltration of immune cells within the TME, suppressing antitumor immunity [18][19][20][21].

2. Collagen Targeting for Anticancer Drug Delivery

The interactions of different proteins with several types of collagen are mediated by specific collagen-binding domains (CBD) [22]. Engineering antibodies, drugs, or cytokines with a CBD allows for the targeting and release of the CBD-associated biomolecules into the tumor collagen scaffold, reducing off-target effects, decreasing toxicity upon systemic administration, and increasing localized retention, thereby enhancing their therapeutic efficacy [23]. As an example, Liang et al. demonstrated that the fusion of a recombinant protein containing the EGFR binding fragment of cetuximab with a CBD results in specific targeting and improved penetration into squamous carcinoma A431 cell xenografts [24]. A similar strategy was used to obtain CBD conjugation to immune checkpoint inhibitor antibodies and fusion to interleukin-2 (IL-2) [25]. Interestingly, in different tumor models, both CBD-fused IL2 and CBD-conjugated checkpoint inhibitors showed enhanced antitumor efficacy and reduced associated toxicity compared with their unmodified counterparts. Moreover, CBD fusion to IL-12 has been described as resulting in systemic toxicity reduction and synergy with immune checkpoint inhibitor therapy [26]. Improvements in the efficacy of cytokine therapy for cancer treatment were also achieved by fusing IL-2 and IL-12 to the collagen-binding protein lumican to potentiate cytokine specificity and local retention and reduce systemic toxicity [27]. Among the strategies to ameliorate in situ drug delivery, the combination of collagen-derived hydrogels and CBD has also been investigated; for instance, localized and controlled delivery of immunotherapeutics has been achieved by the implantation of a collagen hydrogel loaded with interferon-alpha 2b fused to a collagen-binding domain [28]. The above-mentioned studies collectively demonstrate the possibility of targeting both cytokines and immune checkpoint inhibitors by engineering them with collagen-binding peptides or proteins to achieve improved immunotherapy safety and efficacy.

Some recent investigations showed that albumin, the most abundant plasma protein, can also be used as a carrier to improve the pharmacokinetics, solubility and serum stability of anticancer drugs [29]. Moreover, albumin accumulates in the TME since it is used as an energy source by fast-growing cancer cells [30]. Exploiting these favorable pharmacological features, Sasaki et al. developed a strategy to obtain collagen-binding serum albumin drug conjugates. In particular, doxorubicin was conjugated with albumin fused with a CBD and used to treat a murine model of colon carcinoma. In combination with an anti–PD-1 checkpoint inhibitor, the treatment resulted in complete tumor regression by virtue of the significantly higher doxorubicin accumulation observed within the TME [31]. Chemotherapeutic drug delivery by anti-collagen 4 immunoconjugates has also been described as a strategy for tumor stroma targeting [32].

Other than maximizing the therapeutic efficacy of anticancer drugs, tumor collagen drug-targeting has also been considered for enhancing the pharmacokinetics of diagnostic compounds [23]. For instance, the development of a collagen-targeted MRI contrast agent has been described to achieve high sensitivity at low dosage, reduce metal toxicity, and facilitate disease progression monitoring and the early detection of liver metastasis [33][34].

3. Strategies to Promote Tumor Collagen Degradation

3.1. Collagenase Treatment

The administration of different matrix-modulating enzymes, including collagenase, relaxin and hyaluronidase, has been used to promote the degradation of the extracellular matrix (ECM) components aiming at tumor stiffness reduction [35]. In particular, studies performed on animal models indicate that collagenase treatment can improve the diffusion and the uptake of therapeutic macromolecules, nanoparticles, and gene therapy vectors into solid tumors by approximately 2-fold on average [36][37][38]. The clinical significance of this relatively modest effect is controversial, and it is likely dependent on the type and on the stage of the tumor, the delivery route and the duration of the treatment [35][37]. Furthermore, the products of collagen degradation can still promote cancer angiogenesis and metastasis [39]. Therefore, toxicity and immunogenicity of administered collagenase, off-target effects on non-tumor tissues and the possible increase in the tumor metastasis potential need to be precisely addressed before clinical translation.

3.2. Collagenase Encapsulated Nanoparticles and Hydrogels

Advances in nanotechnology and the engineering of hydrogel materials have provided new opportunities for controlled local delivery of ECM-degrading enzymes. Collagenase functionalization of nanoparticles has been shown to promote the degradation of extracellular stroma in different tumor experimental models, thereby enhancing the permeability and retention of antitumor drugs [40][41][42][43]. Pan et al. described a localized co-delivery strategy into HER2-positive BT474 tumor-bearing mice of collagenase and trastuzumab by using a thermosensitive hydrogel, suggesting that this delivery route may promote the penetration of the therapeutic antibody into deeper tumor tissues [44].

3.3. Protein-Free Collagen Degradation

As a strategy to promote the degradation of tumor collagen without the use of collagen-degrading enzymes, Dong et al. described the use of nanoparticles loaded with a chemotherapeutic agent, doxorubicin, and a nitric oxide (NO) donor. The loaded NO induced the activation of resident matrix metalloproteinases (MMPs) that degrade the collagen in the TME, further facilitating the penetration of the nanoparticles and their therapeutic payload in the orthotopic 4T1 breast cancer model [45]. Differently from the use of collagen-degrading enzymes, this alternative strategy leads to increased tumor penetration of both the loaded cargo and the nanoparticle, thus leading to improved anticancer efficacy with reduced toxicity.

3.4. Collagen-Degrading Bacteria

Motile bacteria are promising anticancer drug delivery vectors by virtue of their proteolytic activity toward ECM components that promote solid tumor colonization [46][47]. Recently, the engineering of bacteria to promote ECM degradation has been proposed as an innovative strategy to modify the immune landscape of the TME. In particular, engineered collagen I-degrading *Salmonella typhimurium* effectively targets collagen within the pancreatic ductal adenocarcinoma (PDAC) tissue, reduces the frequency of suppressive intratumoral cells and improves the efficacy of combined immunotherapy treatments [48].

3.5. Degradation of Tumor Extracellular Matrix Mediated by Armed Oncolytic Virus

Oncolytic viruses (OVs) can specifically replicate in tumor cells inducing their lysis; moreover, OVs may also target tumor stromal cells, including cancer-associated fibroblasts (CAFs), leading to profound alterations within the TME [49][50]. As OV engineering allows for the expression of transgenes that may enhance the antitumor immune responses and the TME remodeling, oncolytic adenoviral viruses expressing relaxin [51][52], decorin [53][54][55][56] and MMP-8 [57] have been generated to decrease the synthesis or promote the degradation of components of the ECM, including collagen fibers, thus supporting the viral spread and, consequently, improving virotherapy therapeutic efficacy [58]. Recently, Zhang et al. observed a synergistic antitumor effect of the combination between an oncolytic adenovirus carrying decorin with a CAR T cell therapy targeting carbonic anhydrase IX. In particular, in a xenograft model of human renal carcinoma, this combined therapy altered the distribution of collagen fibers within the TME, promoting the efficacy of CAR T cells by enhancing T cell persistence [59]. Oncolytic viruses have also been engineered to produce bispecific T cell engagers (BiTEs) to target some tumor stromal components to promote antitumor effects [60][61][62].

References

1. Winkler, J.; Abisoye-Ogunniyan, A.; Metcalf, K.J.; Werb, Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nat. Commun. 2020, 11, 5120.
2. Huang, J.; Zhang, L.; Wan, D.; Zhou, L.; Zheng, S.; Lin, S.; Qiao, Y. Extracellular matrix and its therapeutic potential for cancer treatment. Signal Transduct. Target. Ther. 2021, 6, 1–24.
3. Kielty, C.M.; Grant, M.E. The collagen family: Structure, assembly, and organization in the extracellular matrix. In Connective tissue and Its Heritable Disorders; Royce, P.M., Steinmann, B., Eds.; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2002.
4. Bella, J.; Hulmes, D.J.S. Fibrillar collagens. In Fibrous Proteins: Structures and Mechanisms; Springer: Cham, Switzerland, 2017; pp. 457–490.
5. Fuller, A.M.; Eisinger-Mathason, T.S.K. Context matters: Response heterogeneity to collagen-targeting approaches in desmoplastic cancers. Cancers 2022, 14, 3132.
6. Nissen, N.I.; Karsdal, M.; Willumsen, N. Collagens and cancer associated fibroblasts in the reactive stroma and its relation to Cancer biology. J. Exp. Clin. Cancer Res. 2019, 38, 115.

7. Popova, N.V.; Jücker, M. The functional role of extracellular matrix proteins in cancer. Cancers 2022, 14, 238.
8. Peng, D.H.; Rodriguez, B.L.; Diao, L.; Chen, L.; Wang, J.; Byers, L.A.; Wei, Y.; Chapman, H.A.; Yamauchi, M.; Behrens, C.; et al. Collagen promotes anti-PD-1/PD-L1 resistance in cancer through LAIR1-dependent CD8+ T cell exhaustion. Nat. Commun. 2020, 11, 4520.
9. Klemm, F.; Joyce, J.A. Microenvironmental regulation of therapeutic response in cancer. Trends Cell Biol. 2014, 25, 198–213.
10. Angel, P.M.; Zambrzycki, S.C. Predictive value of collagen in cancer. Adv. Cancer Res. 2022, 154, 15–45.
11. Song, K.; Yu, Z.; Zu, X.; Li, G.; Hu, Z.; Xue, Y. Collagen remodeling along cancer progression providing a novel opportunity for cancer diagnosis and treatment. Int. J. Mol. Sci. 2022, 23, 10509.
12. Han, W.; Chen, S.; Yuan, W.; Fan, Q.; Tian, J.; Wang, X.; Chen, L.; Zhang, X.; Wei, W.; Liu, R.; et al. Oriented collagen fibers direct tumor cell intravasation. Proc. Natl. Acad. Sci. USA 2016, 113, 11208–11213.
13. Ray, A.; Callaway, M.K.; Rodríguez-Merced, N.J.; Crampton, A.L.; Carlson, M.; Emme, K.B.; Ensminger, E.A.; Kinne, A.A.; Schrope, J.H.; Rasmussen, H.R.; et al. Stromal architecture directs early dissemination in pancreatic ductal adenocarcinoma. JCI Insight 2022, 7, e150330.
14. Jensen, C.; Nissen, N.I.; Von Arenstorff, C.S.; Karsdal, M.A.; Willumsen, N. Serological assessment of collagen fragments and tumor fibrosis may guide immune checkpoint inhibitor therapy. J. Exp. Clin. Cancer Res. 2021, 40, 326.
15. Wu, F.; Yang, J.; Liu, J.; Wang, Y.; Mu, J.; Zeng, Q.; Deng, S.; Zhou, H. Signaling pathways in cancer-associated fibroblasts and targeted therapy for cancer. Signal Transduct. Target. Ther. 2021, 6, 218.
16. Vannucci, L. Stroma as an active player in the development of the tumor microenvironment. Cancer Microenviron. 2014, 8, 159–166.
17. Sahai, E.; Astsaturov, I.; Cukierman, E.; DeNardo, D.G.; Egeblad, M.; Evans, R.M.; Fearon, D.; Greten, F.R.; Hingorani, S.R.; Hunter, T.; et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat. Rev. Cancer 2020, 20, 174–186.
18. Costa, A.; Kieffer, Y.; Scholer-Dahirel, A.; Pelon, F.; Bourachot, B.; Cardon, M.; Sirven, P.; Magagna, I.; Fuhrmann, L.; Bernard, C.; et al. Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. Cancer Cell 2018, 33, 463–479.e10.
19. Barrett, R.L.; Puré, E. Cancer-associated fibroblasts and their influence on tumor immunity and immunotherapy. eLife 2020, 9, e57243.
20. Peng, L.; Wang, D.; Han, Y.; Huang, T.; He, X.; Wang, J.; Ou, C. Emerging role of cancer-associated fibroblasts-derived exosomes in tumorigenesis. Front. Immunol. 2022, 12, 795372.
21. Mhaidly, R.; Mechta-Grigoriou, F. Fibroblast heterogeneity in tumor micro-environment: Role in immunosuppression and new therapies. Semin. Immunol. 2020, 48, 101417.
22. I Pareti, F.; Fujimura, Y.; A Dent, J.; Holland, L.Z.; Zimmerman, T.S.; Ruggeri, Z.M. Isolation and characterization of a collagen binding domain in human von Willebrand factor. J. Biol. Chem. 1986, 261, 15310–15315.
23. Wahyudi, H.; Reynolds, A.A.; Li, Y.; Owen, S.C.; Yu, S.M. Targeting collagen for diagnostic imaging and therapeutic delivery. J. Control. Release 2016, 240, 323–331.
24. Liang, H.; Li, X.; Wang, B.; Chen, B.; Zhao, Y.; Sun, J.; Zhuang, Y.; Shi, J.; Shen, H.; Zhang, Z.; et al. A collagen-binding EGFR antibody fragment targeting tumors with a collagen-rich extracellular matrix. Sci. Rep. 2016, 6, 18205.
25. Ishihara, J.; Ishihara, A.; Sasaki, K.; Lee, S.S.-Y.; Williford, J.-M.; Yasui, M.; Abe, H.; Potin, L.; Hosseinchi, P.; Fukunaga, K.; et al. Targeted antibody and cytokine cancer immunotherapies through collagen affinity. Sci. Transl. Med. 2019, 11, eaau3259.
26. Mansurov, A.; Ishihara, J.; Hosseinchi, P.; Potin, L.; Marchell, T.M.; Ishihara, A.; Williford, J.-M.; Alpar, A.T.; Raczy, M.M.; Gray, L.T.; et al. Collagen-binding IL-12 enhances tumour inflammation and drives the complete remission of established immunologically cold mouse tumours. Nat. Biomed. Eng. 2020, 4, 531–543.
27. Momin, N.; Mehta, N.K.; Bennett, N.R.; Ma, L.; Palmeri, J.R.; Chinn, M.M.; Lutz, E.A.; Kang, B.; Irvine, D.J.; Spranger, S.; et al. Anchoring of intratumorally administered cytokines to collagen safely potentiates systemic cancer immunotherapy. Sci. Transl. Med. 2019, 11, eaaw2614.
28. Hu, J.-G.; Pi, J.-K.; Jiang, Y.-L.; Liu, X.-F.; Li-Ling, J.; Xie, H.-Q. Collagen hydrogel functionalized with collagen-targeting IFNA2b shows apoptotic activity in nude mice with xenografted tumors. ACS Biomater. Sci. Eng. 2018, 5, 272–282.
29. Cho, H.; Jeon, S.I.; Ahn, C.-H.; Shim, M.K.; Kim, K. Emerging albumin-binding anticancer drugs for tumor-targeted drug delivery: Current understandings and clinical translation. Pharmaceutics 2022, 14, 728.

30. Rahimizadeh, P.; Yang, S.; Lim, S.I. Albumin: An emerging opportunity in drug delivery. Biotechnol. Bioprocess Eng. 2020, 25, 985–995.

31. Sasaki, K.; Ishihara, J.; Ishihara, A.; Miura, R.; Mansurov, A.; Fukunaga, K.; Hubbell, J.A. Engineered collagen-binding serum albumin as a drug conjugate carrier for cancer therapy. Sci. Adv. 2019, 5, eaaw6081.

32. Yasunaga, M.; Manabe, S.; Tarin, D.; Matsumura, Y. Cancer-stroma targeting therapy by cytotoxic immunoconjugate bound to the collagen 4 network in the tumor tissue. Bioconjugate Chem. 2011, 22, 1776–1783.

33. Salarian, M.; Yang, H.; Turaga, R.C.; Tan, S.; Qiao, J.; Xue, S.; Gui, Z.; Peng, G.; Han, H.; Mittal, P.; et al. Precision detection of liver metastasis by collagen-targeted protein MRI contrast agent. Biomaterials 2019, 224, 119478.

34. Salarian, M.; Turaga, R.C.; Xue, S.; Nezafati, M.; Hekmatyar, K.; Qiao, J.; Zhang, Y.; Tan, S.; Ibhagui, O.Y.; Hai, Y.; et al. Early detection and staging of chronic liver diseases with a protein MRI contrast agent. Nat. Commun. 2019, 10, 4777.

35. Hauge, A.; Rofstad, E.K. Antifibrotic therapy to normalize the tumor microenvironment. J. Transl. Med. 2020, 18, 1–11.

36. Dolor, A.; Szoka, F.C. Digesting a path forward: The utility of collagenase tumor treatment for improved drug delivery. Mol. Pharm. 2018, 15, 2069–2083.

37. García-Olmo, D.; Campos, P.V.; Barambio, J.; Gomez-Heras, S.G.; Vega-Clemente, L.; Olmedillas-Lopez, S.; Guadalajara, H.; Garcia-Arranz, M. Intraperitoneal collagenase as a novel therapeutic approach in an experimental model of colorectal peritoneal carcinomatosis. Sci. Rep. 2021, 11, 503.

38. Eikenes, L.; Bruland, S.; Brekken, C.; Davies, C.D.L. Collagenase increases the transcapillary pressure gradient and improves the uptake and distribution of monoclonal antibodies in human osteosarcoma xenografts. Cancer Res. 2004, 64, 4768–4773.

39. Xu, S.; Xu, H.; Wang, W.; Li, S.; Li, H.; Li, T.; Zhang, W.; Yu, X.; Liu, L. The role of collagen in cancer: From bench to bedside. J. Transl. Med. 2019, 17, 309.

40. Murty, S.; Gilliland, T.M.; Qiao, P.; Tabtieng, T.; Higbee-Dempsey, E.; Al Zaki, A.; Puré, E.; Tsourkas, A. Nanoparticles functionalized with collagenase exhibit improved tumor accumulation in a murine xenograft model. Part. Part. Syst. Charact. 2014, 31, 1307–1312.

41. Zinger, A.; Koren, L.; Adir, O.; Poley, M.; Alyan, M.; Yaari, Z.; Noor, N.; Krinsky, N.; Simon, A.; Gibori, H.; et al. Collagenase nanoparticles enhance the penetration of drugs into pancreatic tumors. ACS Nano 2019, 13, 11008–11021.

42. Xu, F.; Huang, X.; Wang, Y.; Zhou, S. A size-changeable collagenase-modified nanoscavenger for increasing penetration and retention of nanomedicine in deep tumor tissue. Adv. Mater. 2020, 32, e1906745.

43. Wang, X.; Luo, J.; He, L.; Cheng, X.; Yan, G.; Wang, J.; Tang, R. Hybrid pH-sensitive nanogels surface-functionalized with collagenase for enhanced tumor penetration. J. Colloid Interface Sci. 2018, 525, 269–281.

44. Pan, A.; Wang, Z.; Chen, B.; Dai, W.; Zhang, H.; He, B.; Wang, X.; Wang, Y.; Zhang, Q. Localized co-delivery of collagenase and trastuzumab by thermosensitive hydrogels for enhanced antitumor efficacy in human breast xenograft. Drug Deliv. 2018, 25, 1495–1503.

45. Dong, X.; Liu, H.-J.; Feng, H.-Y.; Yang, S.-C.; Liu, X.-L.; Lai, X.; Lu, Q.; Lovell, J.F.; Chen, H.-Z.; Fang, C. Enhanced drug delivery by nanoscale integration of a nitric oxide donor to induce tumor collagen depletion. Nano Lett. 2019, 19, 997–1008.

46. Shirai, H.; Tsukada, K. Bacterial proteolytic activity improves drug delivery in tumors in a size, pharmacokinetic, and binding affinity dependent manner—A mechanistic understanding. J. Control. Release 2020, 321, 348–362.

47. Lou, X.; Chen, Z.; He, Z.; Sun, M.; Sun, J. Bacteria-mediated synergistic cancer therapy: Small microbiome has a big hope. Nano-Micro Lett. 2021, 13, 37.

48. Ebelt, N.; Zamloot, V.; Zuniga, E.; Passi, K.; Sobocinski, L.; Young, C.; Blazar, B.; Manuel, E. Collagenase-expressing Salmonella targets major collagens in pancreatic cancer leading to reductions in immunosuppressive subsets and tumor growth. Cancers 2021, 13, 3565.

49. Achard, C.; Surendran, A.; Wedge, M.-E.; Ungerechts, G.; Bell, J.; Ilkow, C.S. Lighting a fire in the tumor microenvironment using oncolytic immunotherapy. EBioMedicine 2018, 31, 17–24.

50. Everts, A.; Bergeman, M.; McFadden, G.; Kemp, V. Simultaneous tumor and stroma targeting by oncolytic viruses. Biomedicines 2020, 8, 474.

51. Kim, J.-H.; Lee, Y.-S.; Kim, H.; Huang, J.-H.; Yoon, A.-R.; Yun, C.-O. Relaxin expression from tumor-targeting adenoviruses and its intratumoral spread, apoptosis induction, and efficacy. JNCI: J. Natl. Cancer Inst. 2006, 98, 1482–1493.

52. Jung, K.H.; Choi, I.-K.; Lee, H.-S.; Yan, H.H.; Son, M.K.; Ahn, H.M.; Hong, J.; Yun, C.-O.; Hong, S.-S. Oncolytic adenovirus expressing relaxin (YDC002) enhances therapeutic efficacy of gemcitabine against pancreatic cancer. Cancer Lett. 2017, 396, 155–166.
53. Choi, I.-K.; Lee, Y.-S.; Yoo, J.Y.; Yoon, A.-R.; Kim, H.; Kim, D.-S.; Seidler, D.G.; Kim, J.-H.; Yun, C.-O. Effect of decorin on overcoming the extracellular matrix barrier for oncolytic virotherapy. Gene Ther. 2009, 17, 190–201.
54. Li, Y.; Hong, J.; Oh, J.-E.; Yoon, A.-R.; Yun, C.-O. Potent antitumor effect of tumor microenvironment-targeted oncolytic adenovirus against desmoplastic pancreatic cancer. Int. J. Cancer 2017, 142, 392–413.
55. Zhang, W.; Zhang, C.; Tian, W.; Qin, J.; Chen, J.; Zhang, Q.; Fang, L.; Zheng, J. Efficacy of an oncolytic adenovirus driven by a chimeric promoter and armed with decorin against renal cell carcinoma. Hum. Gene Ther. 2020, 31, 651–663.
56. Oh, E.; Choi, I.-K.; Hong, J.; Yun, C.-O. Oncolytic adenovirus coexpressing interleukin-12 and decorin overcomes Treg-mediated immunosuppression inducing potent antitumor effects in a weakly immunogenic tumor model. Oncotarget 2016, 8, 4730–4746.
57. Cheng, J.; Sauthoff, H.; Huang, Y.; I Kutler, D.; Bajwa, S.; Rom, W.; Hay, J.G. Human matrix metalloproteinase-8 gene delivery increases the oncolytic activity of a replicating adenovirus. Mol. Ther. 2007, 15, 1982–1990.
58. Wan, P.K.-T.; Ryan, A.J.; Seymour, L.W. Beyond cancer cells: Targeting the tumor microenvironment with gene therapy and armed oncolytic virus. Mol. Ther. 2021, 29, 1668–1682.
59. Zhang, C.; Fang, L.; Wang, X.; Yuan, S.; Li, W.; Tian, W.; Chen, J.; Zhang, Q.; Zhang, Y.; Zhang, Q.; et al. Oncolytic adenovirus-mediated expression of decorin facilitates CAIX-targeting CAR-T therapy against renal cell carcinoma. Mol. Ther. Oncolytics 2021, 24, 14–25.
60. Freedman, J.D.; Duffy, M.R.; Lei-Rossmann, J.; Muntzer, A.; Scott, E.M.; Hagel, J.; Campo, L.; Bryant, R.J.; Verrill, C.; Lambert, A.; et al. An oncolytic virus expressing a T-cell engager simultaneously targets cancer and immunosuppressive stromal cells. Cancer Res. 2018, 78, 6852–6865.
61. De Sostoa, J.; Fajardo, C.A.; Moreno, R.; Ramos, M.D.; Farrera-Sal, M.; Alemany, R. Targeting the tumor stroma with an oncolytic adenovirus secreting a fibroblast activation protein-targeted bispecific T-cell engager. J. Immunother. Cancer 2019, 7, 19.
62. Heidbuechel, J.P.W.; Engeland, C.E. Oncolytic viruses encoding bispecific T cell engagers: A blueprint for emerging immunovirotherapies. J. Hematol. Oncol. 2021, 14, 63.