A Senescence-Targeting and Activatable NIR-II Theranostic Nanoparticle for Photodynamic Therapy of Degenerative Tendinopathy in Rats

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ABSTRACT INTRODUCTION: Senescent cells (SnCs) are a key pathological driver in degenerative tendinopathy, contributing to pain, dysfunction, and failed healing. However, the inability to precisely target and eliminate SnCs within the complex tendon milieu remains a major challenge. Our objective was to develop a precision nanomedicine that integrates local delivery, specific senescent cell detection, and spatially controlled elimination for the treatment of degenerative tendinopathy.

METHODS: We designed a novel theranostic agent comprising a near-infrared-II (NIR-II) fluorescent photosensitizer quenched by a galactose moiety, which serves as a specific substrate for senescence-associated β-galactosidase (SA-β-gal). This prodrug was encapsulated into liposomes, and the surface was functionalized with a peptide that specifically binds to senescent cell membrane markers. The final construct, termed TPA-T-βGal, was characterized for its physicochemical properties. In vitro, the effect of TPA-T-βGal-mediated PDT on cellular senescence and differentiation lineage commitment was assessed in primary tendon stem/progenitor cells (TSPCs). For in vivo evaluation, a rat model of degenerative Achilles tendinopathy was established. Animals received a local peritendinous injection of TPA-T-βGal or control particles. NIR-II fluorescence imaging was performed to non-invasively map SnC distribution and probe retention. Subsequently, the diseased tendon was irradiated with an 808 nm laser to activate the photosensitizer for photodynamic therapy (PDT). Therapeutic outcomes were comprehensively assessed through histopathology (H&E, Masson's Trichrome, Alcian Blue, Safranin O/Fast Green), micro-computed tomography (micro-CT) for ectopic ossification, gait analysis, and tendon mechanical failure testing. All animal procedures were approved by our Institutional Animal Care and Use Committee (IACUC).

RESULTS SECTION: TPA-T-βGal demonstrated high specificity for SnCs in vitro, with SA-β-gal-triggered NIR-II fluorescence activation and potent PDT effect. Critically, in TSPC cultures, TPA-T-βGal-PDT effectively eliminated SnCs and reshaped the cellular microenvironment, resulting in a rejuvenated TSPC population with a significantly enhanced capacity for tenogenic differentiation, while suppressing undesirable osteogenic and chondrogenic differentiation pathways. Following local injection in the rat tendinopathy model, NIR-II imaging confirmed strong local retention and activation of TPA-T-βGal at the tendon site. The TPA-T-βGal+PDT group exhibited a significant reduction in SnC burden compared to controls. This targeted senolysis led to comprehensive therapeutic benefits: histopathological analysis revealed better tissue architecture restoration, with reduced glycosaminoglycan depletion and collagen disarray. Micro-CT quantification showed a significant decrease in ectopic ossification volume. Functionally, rats in the treatment group demonstrated superior weight-bearing and gait patterns. Most importantly, tendon failure testing confirmed a statistically significant increase in the ultimate failure load and stiffness, indicating robustly enhanced tendon strength.

DISCUSSION: We successfully developed a multifunctional nanoparticle, TPA-T-βGal, that achieves dual-targeting to SnCs in tendinopathy through peptide-mediated binding and SA-β-gal-specific activation. Local administration ensures high therapeutic concentration at the disease site. The subsequent PDT enables precise, light-triggered ablation of SnCs. Our in vitro data establish a fundamental mechanism: by removing SnCs, TPA-T-βGal-PDT not only halts detrimental processes but actively promotes a pro-regenerative niche by steering TSPCs toward a functional tenogenic lineage, thereby directly countering the pathological ossification and chondrogenesis seen in tendinopathy. This cellular reprogramming underpins the in vivo outcomes: the reversal of abnormal matrix composition, ectopic ossification, and functional impairment, culminating in restored tendon mechanical strength. We conclude that the locally delivered TPA-T-βGal represents a potent and comprehensive "see-and-treat" strategy for precise senolysis and functionally-directed tendon regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: This study presents a novel and clinically relevant theranostic strategy. The local injection approach mirrors a potential clinical application for tendon treatments. By not only clearing senescent cells but also actively promoting a pro-regenerative cellular state and correct differentiation path in TSPCs, this strategy moves beyond mere cytoreduction to true mechanism-guided tissue regeneration, offering high translational potential for restoring function in degenerative tendon diseases.

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