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Exploring a Novel Approach to Senolysis Using a Urokinase Plasminogen Activator Receptor-targeting Antibody-Drug Conjugate

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Abstract. A multitude of highly deleterious age-related diseases are strongly associated with cellular senescence and the accumulation of senescent cells. Senescent cells are defined by a permanent growth arrest and exhibit increasing amounts of harmful characteristics as people age. Removing senescent cells through senolysis reduces the likelihood of developing senescence-associated pathologies. Through antibody-drug conjugates (ADCs), targeted senolysis that ablates senescent cells while causing minimal damage to healthy tissues can be achieved. Urokinase plasminogen activator receptor (uPAR) is an abundant protein in the surfaceome of diverse types of senescent cells and an established biomarker for cellular senescence. This study proposes a novel ADC-based senolytic strategy by designing an ADC tailored for senolysis that targets uPAR. By leveraging both uPAR overexpression as well as the upregulation of an internalization mediator — plasminogen activator inhibitor-1 (PAI-1) in senescent cells. The design seeks to maximize therapeutic efficacy for preventing various age-related diseases while bearing minimal off-target risks.

Keywords: urokinase plasminogen activator receptor, antibody-drug conjugate, cellular senescence, senolysis, biological marker

1. Introduction

Cellular senescence commences in response to endogenous and exogenous stress signals such as DNA damage, the shortening of telomeres, organelle stress, and oncogene activation [1]. A senescent cell is characterized by its inability to proliferate indefinitely despite being present in optimum growth conditions with mitogenic signals [1]. At an early age, senescence acts as a stress response that comes with a variety of beneficial effects, such as guiding embryonic development and wound healing [1]. Senescence is also known for its role in tumor suppression, as this process aids in the prevention of the propagation of aberrant or damaged cells [1]. However, being an antagonistic pleiotropic process, cellular senescence comes with many deleterious properties that show as people age [1,2]. Studies have shown that the accumulation of senescent cells facilitates the development of a plethora of significantly pernicious, age-related pathologies such as Alzheimer's disease, atherosclerosis, and Parkinson's disease [1,2]. The ablation of senescent cells (termed senolysis) using molecular agents referred to as senolytics hinders the development of senescence-

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associated pathologies, thus alleviating the symptoms of various age-related dysfunctions [1,3]. Senescent cells display altered metabolic profiles and are associated with a variety of distinct biomarkers, some of which are shown to be prominent targets for senolytics [1]. Urokinase plasminogen activator receptor (uPAR) binds with urokinase-type plasminogen activator and regulates the proteolysis of the extracellular matrix (ECM) [4]. The elevated expression in tumor cells has been implicated to be instrumental to tumor motility [4]. It has been observed that the expression for uPAR is significantly upregulated in various types of senescent cells, contrasting the sparing uPAR expression in vital organs [3,5]. The substantial difference between the uPAR expression level in senescent cells and most functional cells warrants it to be studied as a potential target for senolysis.

An antibody-drug conjugate (ADC) is a conjugate of an antibody and small cytotoxic molecular payloads linked via linker molecules [6]. Studies have shown that ADCs act as potent senolytics due to their high targetability and potent cytotoxic properties [7]. A recent study yielded results that validated the potency of uPAR as a target for ADC-based treatments by utilizing a uPAR-ADC to target pancreatic ductal adenocarcinoma (PDAC) cells [8]. This study proposes a uPAR-ADC-mediated approach to senolysis along with a senolytic uPAR-ADC design [8]. The approach to ADC design in this study diverges from the ADCs being developed for cancer treatment.

2. Current landscape of senolysis

Some approaches to senolysis that are popular today include the exploitation of the elevated senescence-associated β-galactosidase (SA-β-gal) activity as a route for drug delivery and targeting upregulated negative modulators for apoptosis, such as members of the BCL-2 family, using senolytics that inhibit the functions of said modulators [1].

Chimeric antigen receptor (CAR) T cells-based and ADC-based approaches to senolysis have shown potency when it comes to eliminating senescent cells [3,5,7]. However, these techniques have yet to be intensively explored as means of senolysis. Insights into integrating these novel strategies into senolytic therapy prove crucial for the advancement of the field.

3. Characterizing uPAR-ADC-based senolysis

Targeting uPAR on the surface of senescent cells comes with a set of unique challenges that needs to be tackled through meticulous design of the ADC. First, uPARs can be proteolytically cleaved in response to pro-inflammatory signals. Such cleavage produces soluble urokinase plasminogen activator receptors (suPARs) that circulate in the bloodstream [9]. The concentration of suPAR elevates with age and is a part of the senescence-associated secretory phenotype (SASP) [9]. The presence of these uPAR analogs may act as decoys for the membrane-bound uPARs, causing uPAR-ADCs to bind to them, leading to decreased drug pharmacokinetics (PK), and senolytics carrying cytotoxic compounds circulating in the blood. Second, senolytic therapy via ADCs is a systemic treatment that involves the delivery of senolytics to various locales of the body. Should the ADCs cause an excessive and undesirable bystander effect, vital tissues and organs may be damaged as a result. Third, despite in relatively minute amounts, cell types, including endothelial cells and specific innate immune cells, do exhibit some levels of uPAR expression [10]; navigating the design of a uPAR-ADC to minimize or mitigate harm dealt to these cells is of particular importance.

UPAR does not internalize spontaneously upon binding to uPA [11,12]. However, the internalization process commences once a bound uPA complexes with plasminogen activator inhibitor-1 (PAI-1) [11,12]. As shown in figure 1, this process happens as an internalization mediator

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— low-density lipoprotein receptor-related protein 1 (LRP1) recognizes uPA-PAI-1 complex and initiates clathrin-mediated endocytosis as a result [11]. PAI-1 expression is upregulated in senescent cells [13]. Incorporating this built-in endocytosis mechanism can help achieve a high internalization efficacy.

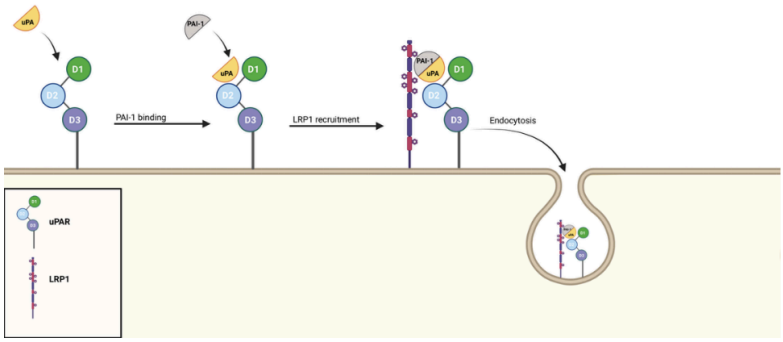

Figure 1. Schematic representation of the key steps involved in the uPA-PAI-1-initiated, LRP1-mediated internalization mechanism. A uPA bound to uPAR associates with PAI-1; the complex then recruits LRP1, which facilitates internalization via endocytosis

4. Design of a senolytic uPAR-ADC

With the aforementioned caveats and exploits in mind, this study proposes to build a uPAR-ADC based on the uPAR-targeting monoclonal antibody (mAB) ATN-658 [14]. Its epitope binds to the D3 domain of uPAR, making the binding of the ADC not sterically compete with uPA, thus enabling the utilization of the aforementioned uPA-PAI-1 mediated internalization route [4,14]. The D3 domain is most proximal to the surface membrane [4]; it is postulated that the possible membrane-induced conformation change to the domain may cause the binding of ATN-658 to favor the anchored uPAR rather than the plasmatic suPAR.

To address the risk of systemically administered ADCs targeting healthy cells with moderate uPAR surface presentation, the uPAR-ADC design should have a high plasma stability and needs to carry a relatively small number of payloads to minimize the cytotoxicity per conjugate. This study aims to use a Val-Cit-PABC cleavable linker to conjugate duocarmycin payloads to the antibody at engineered cysteine sites (one per heavy chain) via stable maleimide-thiol linkages. This yields homogeneous ADCs with a low drug-to-antibody ratio (DAR) of 2, high plasma stability [15], and predictable PK. Following internalization through endocytosis and lysosomal cleavage of linker, the DNA-damaging payloads can then be released to eliminate senescent cells selectively as shown in figure 2.

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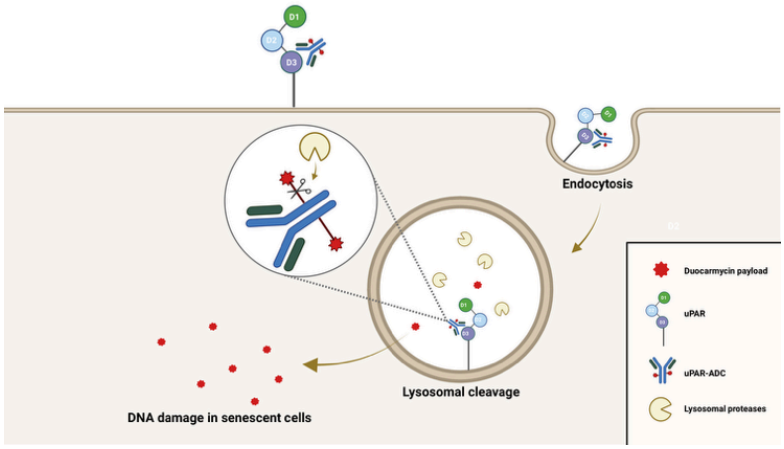

Figure 2. Diagram illustrating the proposed mechanism of action of uPAR-ADC. After binding to the membrane-proximal D3 domain of a uPAR and internalization, the linkers in the ADC are cleaved via lysosomal enzyme activities, releasing duocarmycin payloads for the downstream elimination of senescent cells

5. Discussion

Recent studies have shown that uPAR is a promising target for ADCs [8]. Senolytic anti-uPAR CAR T cells have also displayed great efficacy in removing senescent cells in mice [3]. These studies provide evidential information that supports the validity of rationale that a uPAR-ADC-based approach towards senolysis can be practical. However, it is crucial to point out that the proposal in this study has yet to undergo experimental validation. Since this study is the first to suggest the use of uPAR-ADC in senolysis, this theory remains hypothetical. Although evidence have ensured that the use of senolytic uPAR-ADC discussed in this study to combat cellular senescence is theoretically viable and potent, unforeseen factors that render the idea invalid, such as a higher level of bystander effect or unanticipated off-target toxicity, may arise during in vitro and in vivo experiments. If such factors arise, iterative optimization of the ADC design would be called for.

6. Conclusion

This study discussed a novel use of a uPAR-targeting ADC to tackle cellular senescence. The advancing field of ADC technologies holds promises for auspicious strategies to senolysis. Nevertheless, a relatively small number of studies have investigated ADC-based senolytic methods. While other studies have explored applications of ADC-based senolytics targeting various surface biomarkers for senescent cells like B2M, this is the first proposal to explore the potential of uPAR as a target for senolytic ADCs. This study believes that the abundance of uPAR across various types of senescent cells as well as the upregulation of PAI-1 in cellular senescence, which aids the internalization process, mark uPAR as a particularly compelling target for ADCs. This study also encourages further studies to be made on the applicability of this senolytic approach, including experimental validation of uPAR-ADC design and the possibility of combining this study's approach with other senolytic methods to produce an all-encompassing senolysis strategy.

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