Senotherapeutic drugs: A new avenue for skincare?

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Authorship

BPL and LWH contributed to conceptualization, drafting and review of the manuscript.

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Off-Label Use/Unapproved Drugs or Products

Table 1 contains reference to the potential unlabelled repurposing of drugs for senotherapy. We would like to declare that these drugs are still investigational.

Summary

Skin ageing is an outward manifestation of other cellular and molecular ageing processes occurring elsewhere in the body. These processes are known collectively as the 'hallmarks' of ageing, which are a series of basic health maintenance mechanisms that fail over time. Cellular senescence is one of the most studied of the hallmarks of ageing; senescent cells accumulate over time and are major drives of the ageing process. Here, we discuss the impact of cellular senescence in the context of skin ageing, and discuss the emerging landscape of interventions designed for their selective removal by targeted cell death (senolytics) or rejuvenation (senomorphics). We discuss the serotherapeutic strategies that are currently under investigation for systemic ageing which may bring eventual benefits for skin health. Next, we discuss a newly discovered hallmark of ageing, dysregulated mRNA processing, which can be targeted for senomorphic effect. Finally, we highlight a new modality for manipulation of disrupted mRNA processing, oligonucleotide therapeutics. The emerging field of senotherapeutics is set to revolutionise how we view and treat skin ageing, and senotherapies are now poised to become a new class of skincare interventions.

Senescent cells: An important cause of cellular ageing

Systemic cellular ageing occurs because of the failure of a few basic health maintenance mechanisms, which collectively are known as the hallmarks of ageing (1). These are an interconnected set of cellular processes that determine how organs and systems age. One of the hallmarks of ageing is cellular senescence; senescent cells are alive and metabolically active, but non-proliferative. Importantly, they demonstrate differential functions to their native counterparts. Predominant amongst these new characteristics is the secretion of the senescence-associated secretory phenotype (SASP), a collection of pro-inflammatory cytokines and tissue remodelling proteins (2). In young and healthy tissues, senescent cells and their associated SASP has an important role in normal biology, with roles in wound healing, cancer prevention and embryonic development (3). In ageing systems however, the unresolved clearance of even small numbers of senescent cells and their associated SASP response can result in profound changes to the tissues and organs that are characteristic of ageing (4, 5). Selective ablation of senescent cells in transgenic animal models indicated that the removal of senescent cells was able to delay several age-associated diseases (6). Follow on work has since demonstrated that removal of senescent cells yields improvements in renal, cardiac, motor and cognitive functions in animal models (7). Senescent cells thus comprise a tractable and emerging target for new therapies aiming to attenuate ageing phenotypes.

Senescence in the context of skin ageing

The hallmarks of ageing act on skin, as they do on other organs. DNA damage induced by sun exposure can cause the characteristic aesthetic signs of ageing, as can epigenetic changes resulting from exposure to pollutants and other damaging chemicals. Inflammation, arising from dysfunctional cell communication can lead to skin reddening, changes to the extracellular matrix and inflammatory infiltration. Stem cell exhaustion also means that skin tissues may lose their ability to repopulate and differentiate following the loss of cells through damage or senescence. Senescent cells accumulate in

the cells of the epidermis and dermis, as well as in the subdermal adipose tissue depots (figure 1), as they do in all tissues and organs. The secretion of the SASP may also drive aberrant tissue remodelling and extracellular matrix dysfunction, causing changes in collagen composition and structure, destruction of elastin as well as inflammatory infiltration, fibrotic changes and atrophy of fat tissues. Collectively, these phenomena lead to the characteristic aesthetic changes associated with ageing, including rhytids, pigmentation changes, skin thinning and deterioration of the underlying skin substructure. Removal or rejuvenation of senescent cells therefore has the potential to remove the negative effects of the SASP, leading to normalisation of the extracellular matrix, renewed differentiation of new adipocytes, reduction of overt inflammation and restoration of the skin substructure (figure 2). Interventions designed to reduce the senescent cell load of aged skin are amenable for topical delivery to treat the most external layers of the skin to ameliorate pigmentation changes and surface skin quality, as well as having positive effects on skin substructure if delivered by injection to the dermis or the adipose tissue depots.

Senolytics and senomorphics: alternative approaches for the removal of senescent cells

There are two basic approaches for the removal of senescent cells. These are senolytic approaches whereby senescent cells are killed selectively, or senomorphic approaches whereby they are rejuvenated. There may be benefits and drawbacks to both approaches. Rejuvenated senescent cells may require repeated treatment to maintain their renewed status, and will of course retain some features of age, similar to non-senescent cells present in the host. Senolytic approaches however may not take account of findings that senescent cells comprise several subtypes, some of which may be beneficial (8). Removal of senescent cells by selective apoptosis will likely affect both subtypes without discrimination. The necrotic factors and other cell signals released upon cell death associated with proinflammatory mediators and immune responses may also cause tissue damage and contribute to disease pathogenesis (9). Furthermore, some disease indications may involve tissues that are cell

poor, and may not tolerate cell removal. It is likely therefore, that the choice of senotherapeutic modality that is most appropriate in any particular instance will depend on the therapeutic aim. Potential senolytic and senomorphic candidates are given in table 1.

New modalities for the removal or rejuvenation of senescent cells

To date, the majority of senotherapeutic approaches have been based on small molecule candidates. There are however some emerging novel modalities for targeting senescent cells. These include approaches to harness the immune system for clearance of senescent cells. T cells engineered to express chimeric antigen receptors (CAR T therapies) have emerged as a new potential means to clear senescent cells. For example, CAR T cells engineered with the urokinase-type plasminogen activator receptor (uPAR) have been demonstrated to reverse senescence-associated pathologies in animal models (10). Other approaches have involved the use of proteolysis-targeting chimeras (PROTACs), whereby a ligand to a target of interest is conjugated to an E3 ubiquitin ligase, which brings about proteolytic degradation of targets. A PROTAC targeted to BRD4, demonstrated good senolytic activity in cultured cells and animal models (11). Other approaches target the unique characteristics of senescent cells for senotherapeutic purposes. One such property is the very high levels of lysosomal beta galactosidase that are present in senescent cells. Drugs with known senolytic or senomorphic properties can be galactose modified, and can thus be used to produce a prodrug that is only processed to its active form in senescent cells (12).

Targeting RNA processing for senomorphic effect

RNA processing is the collection of events that are necessary to allow the production of multiple mRNAs from a gene, in a process known as alternative splicing (AS). AS is a pre-requisite to the plastic and adaptable transcriptome necessary for avoidance of cellular senescence. The decision as to which

alternative RNA is expressed in any given situation is made by the combinatorial binding of a group of proteins called splicing factors (13). Dysregulation of AS has emerged as a new, and therapeutically tractable, hallmark of ageing (14), and disruption to this is associated with cellular senescence and adverse ageing outcomes *in vitro* and *in vivo* (15-18). Splicing factor expression declines with age as a result of repeated and constitutive activation of the AKT and ERK signalling pathways, and their effector genes FOXO1 and ETV6 (19). A promising new senomorphic strategy for cellular rejuvenation involves the rescue of splicing factor expression by genetic or small molecule means, and restoration of more youthful splicing patterns. Splicing factor expression can be restored by naturally occurring small molecules such as polyphenols (20) or donors of the gasotransmitter hydrogen sulfide (21), or by inhibition of their upstream negative regulators AKT and ERK (19). These interventions result in the rejuvenation of senescent cells and the attenuation of the SASP, with or without rebuilding of telomeres and resumption of cell cycle, depending on the intervention. Importantly, these interventions would not need to be applied daily for reversal of senescence; treatment with polyphenols was shown to provoke a measureable effect on senescent cell load in human primary dermal fibroblasts 4 weeks after initial treatment (22), whereas treatment with H_2S donors was able to retard senescence in human endothelial cell models (21). This raises the possibility of prophylactic application for skin ageing phenotypes.

Oligonucleotide therapies; future precision medicine for cellular senescence

The majority of senotherapeutic candidates available at present have potential off target effects. Manipulation of signalling pathways such as p53, JAK-STAT, ATM or AKT will yield effects on many other downstream targets in addition to those intended. Similarly, small molecules such as fisetin, dasatinib or digoxin may produce unforeseen effects on other cell types or organ systems. Our discovery of the pivotal role of disrupted splicing in cellular senescence raises the possibility of targeting individual splicing events in a very precise manner, which may allow us to pinpoint and target

the exact molecular causes of cellular senescence in the future. Splicing patterns can be modified by the use of splice switching oligonucleotide biologics (SSOs), which bind to the pre-RNA sequences that define splice sites and promote or forbid their usage (23). By these technologies we can either restore the expression of individual splicing factors (since splicing factors are themselves regulated by alternative splicing (24), or force the expression of youthful patterns of splicing for key senescence genes. These emerging approaches should replicate the natural regulatory relationships that maintain homeostasis and molecular stress resilience in young cells, and may form the basis for long-term rejuvenation of aged cells, tissues and organs.

Progress towards the clinic and future outlook

Although evaluation of senotherapeutics is at present in the pre-clinical phase for the majority of indications, several are now entering trials for ageing phenotypes; NCT02848131 (dasatinib and quercetin for the treatment of chronic kidney disease), NCT0367524 (fisetin in the context of frailty), NCT029151898 and NCT 03451006 (metformin, also as an intervention for frailty) (25). The use of senotherapeutics for skin phenotypes is however in its infancy, despite the observation that skin may represent an early human in vivo proof of concept for these approaches, due to its accessibility compared with other organ systems. At the time of writing, the only senotherapeutic intervention on the market specifically for skin aesthetics is IDR-1018, a proprietary innate defence regulatory peptide which has been reported to exhibit some senomodulatory effect (26). The long lasting effect of the novel modalities we describe here opens doors to the development of a new range of clinician dispensed skincare products that could be applied weekly or more frequently in the case of topical application, or at longer intervals by subdermal injection in a clinical dermatology setting.

Conclusion

Cellular senescence is emerging as one of the most tractable intervention points for cellular and organismal ageing. Whilst harnessing these interventions for systemic clinical benefit for the diseases of ageing is some way from the clinic at present, topical or injected application for skin ageing is rather nearer term. Topical application negates many of the barriers associated with systemic toxicity or difficulty in delivery to target organs, whilst providing early human *in vivo* proof of principle for later endeavours. Senotherapeutic approaches to remove or rejuvenate senescent cells offer an 'inside out' approach to ameliorating the aesthetic effects of the ageing process, treating the cause of cellular ageing at its root, rather than managing the effects of the passage of time.

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315 **Table 1: Some examples of senolytic and senomorphic drugs**. The molecular targets and mode of
316 intervention are provided for a non-exhaustive list of senolytic and senomorphic compounds**.** These
317 approaches, although primarily experimental at present, are under exploration for clinical use in some
318 cases.

Senolytic approaches			**Senomorphic approaches**		
Target	**Agent**	**Reference**	**Target**	**Agent**	**Reference**
BCL2 family	pz15227, ABT-263	(27, 28)	NRT1	lamivudine	(29)
			Splicing factors	Resveratrol	(20)
Hsp90	17-DMAG	(30)	Splicing factors	H2S	(21)
MDM2	UBX0101	(31)	SH-6	AKT	(19)
FOXO4	FOXO4-DR1 peptide	(32)	Trametinib	MEK	(19)
USB7	P5091	(33)	NBD peptide	IKK/NFB	(34)
OXR1	Piperlongumine	(35)	ruxolitinib	JAK/STAT	(36)
RTX	Dasatinib	(37)	JH4	LaminA/C	(38)
Na+/K+ ATPase	Digoxin	(39)	ESC-CM	PDGF/FGF pathway	(40)
BRD4	JQ1	(11)	TGFBR2/p21 pathway	miR-291a-3p	(41)
GLS1	PBTES	(42)	ATM	KU-60019	(43)
Fisetin	P13K/AKT	(44)	IDR-1018	Innate defence regulatory peptide	(26)
Panobinostat	HDAC	(45)			
Quercetin-3-D-galactose	multiple	(46)			

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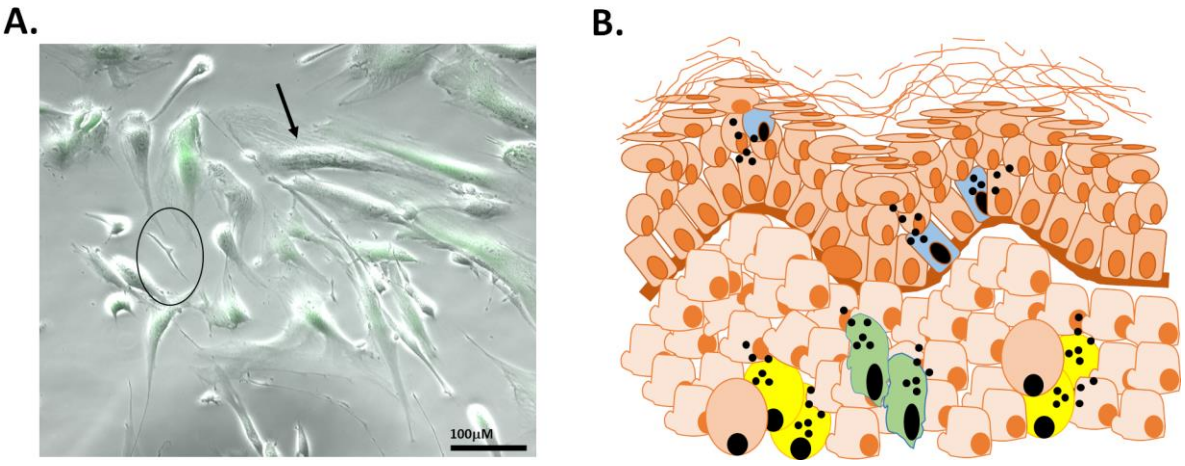
Figure Legends

Figure 1: Senescent cells accumulate in aged skin. A. Senescent human primary dermal fibroblasts in culture. Senescent human dermal fibroblasts stained against senescence–associated beta galactosidase can be seen marked in green in panel A. The black arrow marks a typical senescent cell, which can be identified by its unique morphology consisting of an enlarged, lacy appearance with the presence of multiple vacuoles in addition to its green colour. For comparison, a non-senescent cell in the culture is circled. B. Schematic illustrating the accumulation of senescent cells in all layers of the skin. Senescent keratinocytes are indicated in blue, senescent dermal fibroblasts in green and senescent adipocytes in yellow. Secreted SASP proteins are marked by black dots.

Figure 2: Potential avenues for senotherapeutic approaches to skincare. Young skin (A) is exposed to skin damaging agents over a lifetime including UV light, atmospheric pollutants and oxidative stress, which eventually causes the accumulation of senescent cells in all layers of the skin superstructure, and skin ageing (B). Senotherapeutic agents could be applied in a reactive fashion, either topically for epidermal and upper dermal features, or by injection for dermal and subdermal features, to restore skin to a more youthful state (C). Equally, it is possible that treatments could start prophylactically, in advance of skin ageing phenotypes, to slow or even prevent the age-related deterioration of skin.

340 Figure 1



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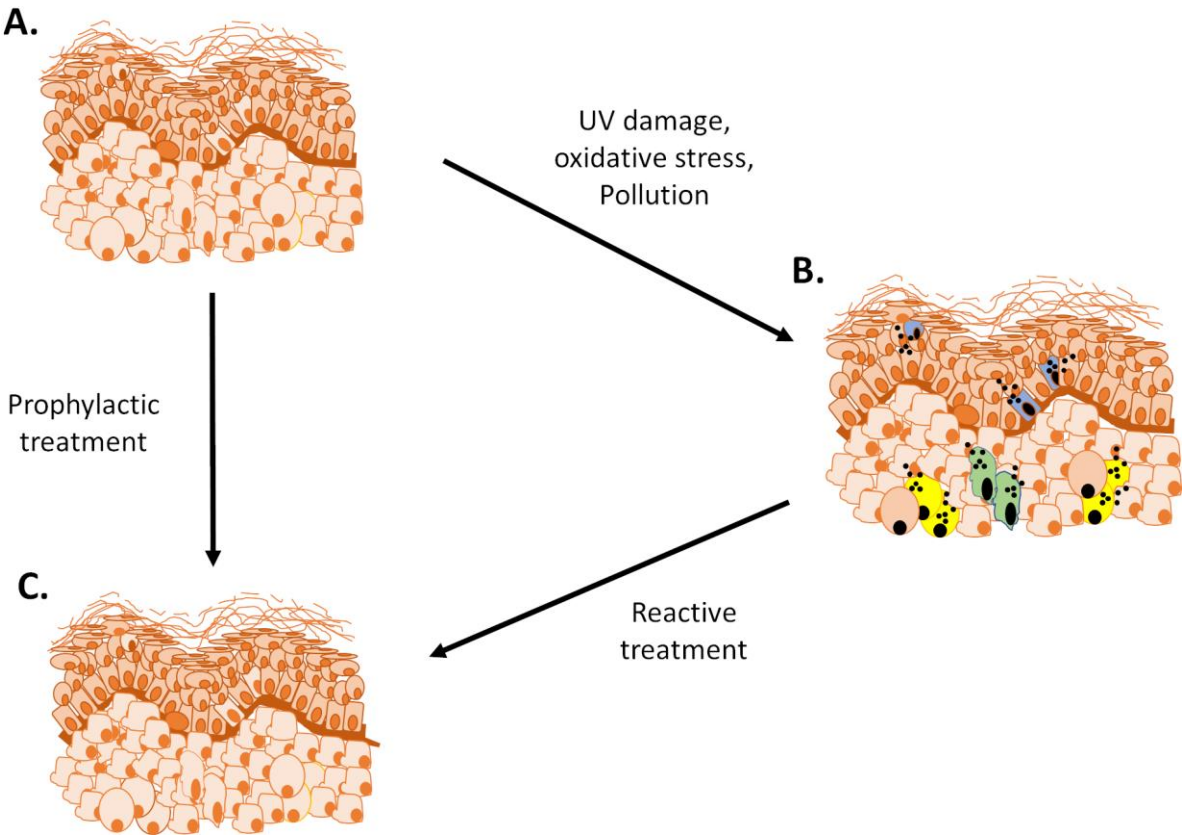
343 Figure 2



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