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Proteomic Revelations

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Summary

The power of proteomics in cultured skin fibroblasts from individuals both systemic sclerosis and recessive dystrophic epidermolysis bullosa has led to the common finding of senescence and deficiencies in autophagy. Both of these disorders exert high demand on fibroblast activity, and without the protective action of autophagy, cellular stress could have many adverse effects that are further amplified by the senescent phenotype.

Pullquote: "the two reports illustrate the power of proteomic analysis as a hypothesis-generating tool that can identify novel pathways associated with important pathologies"

Two recent, related papers in this journal[1, 2] have used a proteomic approach to explore the hypothesis that skin fibroblasts from an immune-mediated, fibrotic disorder (systemic sclerosis; SSc) and a genetic disease epidermo-dermal junction (recessive dystrophic epidermolysis bullosa; RDEB) may share a common features that are associated with the fibrotic/scarring complications of each condition. On the surface, SSc and RDEB represent extremes of cutaneous integrity, in which excessive, immune-mediated dermal activation of fibrogenesis in SSc contrasts with loss of collagen VII and excessive fragility of the dermal-epidermal junction that leads to a continuous cycle of damage and repair. Nonetheless, dermal activity in RDEB frequently leads to mutilating scarring as a consequence of persistent abrasion, blistering, and loss of the epidermis[3].

In the SSc report, Dumit *et al.*[1] conducted a proteomic evaluation of an aging (up to 33y) sequence of dermal fibroblasts with the SILAC technique, in which intracellular proteins from a reference fibroblast population were labeled to steady-state with a mixture of two "heavy" (C^{13},N^{15})-labeled amino acids, paired with separate, individual samples, and then compared for individual protein content, on a relative basis of abundance, by LC-MS-MS. Secreted molecules such as cytokines, matricellular proteins, and matrix proteins were thereby underrepresented. Among the many age-dependent differences that were identified and categorized in the report, the data were used to focus on the decline of members of the

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minichromosome maintenance helicase complex (MCM), particularly on MCM6 and MCM 7, both of which appeared to decline sharply in abundance in skin fibroblasts from donors >30y. Based on a limited sample size, the prevalence of these proteins was correlated with declining cell proliferation, increasing cellular protein content, reduced autophagic flux and several markers of cellular senescence, including accumulation of senescence-associated β-galactosidase (SA-β-gal)[4]. Having validated this approach in cells from normal subjects, a small cohort of SSc fibroblasts were similarly evaluated and shown to share the properties of older donor cells, suggesting that reduced MCM7/autophagy in SSc could contribute to the senescent phenotype. This hypothesis was supported by siRNA knockdown of MCM7 in normal cells. Interestingly accelerated senescence has recently been reported in mesenchymal stem cells from SSc patients[5].

The study of Küttner *et al.*[2], applied the same SILAC strategy to identify consistent proteomic differences between RDEB and age-matched normal dermal fibroblasts. As in the aging/SSc cohorts, the RDEB cells exhibited reduced autophagic flux, reduced proliferative activity, and reduced MCM7 levels, suggesting to these authors that the consequences of defective *COL7A1* generate a negative influence on autophagy and therefore increased levels of cellular stress and cellular senescence. However, the report places greater emphasis on the overabundance of tissue transglutaminase 2 and the possible effects of altered protein crosslinking.

The common features of the two reports are the proteomic technology used to compare human skin fibroblast contents in culture and a common deficit in the clearance of defective organelles and proteins exhibited by cultured fibroblasts from two widely different connective tissue abnormalities that share a scarring phenotype. Although molecular depletion (via siRNA) of MCM7 implicated the protein as a causative agent for increased markers of senescence in normal cells, there were no complementary data to show that overexpression of this marker could rescue all or part of the senescent phenotype in either SSc or RDEB fibroblasts. These findings are certainly thought provoking but not definitive.

Autophagy is generally invoked as a pathway by which cells, through recycling of organelles and other structures, maintain sufficient energy sources to survive physiologic stress and evade apoptosis[6]. Various forms of cellular stress, including reactive oxygen species, result in a rise in protective autophagy and may be accompanied by increased signs of cellular senescence, including lower proliferation, increased cell size, and expression of senescence-associated β-galactosidase. While the authors state that reduced autophagy activity indicates elevated stress (in RDEB), their findings could suggest that inefficient autophagy is the cause, rather than the source, of increased cellular stress. In addition, RDEB fibroblasts in culture exhibited poor adhesion, a factor that could promote anoikis, which is in itself counteracted by autophagy[7].

Fibrosis is an aspect of both SSc and RDEB that may contribute to the apparent senescent phenotype through excessive proliferative demands on resident fibroblasts. Cells from chronic lesions and scars can acquire a senescent phenotype, in which they have a reduced proliferative capacity that has been attributed to the elevated expression of the matricellular protein, CCN1[8]. In turn, the senescent phenotype is also reported to shift fibroblasts to a

proinflammatory, senescence-associated secretory phenotype[9] that can also alter the growth and repair of surrounding tissue. The cytokine cloud associated with senescent cells may itself have effects on autophagy and matrix metabolism. Evaluation of the paracrine effects of SSc and RDEB fibroblasts on normal counterparts could test these mechanisms.

The process of cellular senescence occurs both *in vivo* and *in vitro*, and fibroblasts from (much) older human donors approach that threshold at an earlier passage number. Some would argue that any analysis of serially propagated cells is inherently a selective process in which the most rapidly proliferating subset (or clone) of cells eventually dominates the culture[10]. Indeed, studies of cellular senescence need to carefully control for the number of population doublings. Early- and late-passage cells may well have different cellular phenotypes. For these reasons, studies of senescence-related cell behavior should make every endeavor to report the number of population doublings undergone by the cultures and to correlate *in vitro* findings with intact tissue.

The mechanical environment is another modulator of fibroblast behavior that is gaining in importance as an effector of phenotype. Most fibroblast analyses are performed on rigid plastic culture surfaces that may or may not be coated with specific adhesion molecules. Under these conditions of high (megapascal) rigidity, fibroblasts often spontaneously transform into myofibroblasts as they attempt to match their tensional state with that of the environment. This factor, together with the use of serum-containing media, poses very different, wound-like conditions on the fibroblast. In contrast, these cells grown on softer substrates show very different cytoskeletal and membrane architecture, a different gene expression profile, and proliferative activity. Although culture on plastic is a consistent condition, it may be far from physiological.

Despite the limitations of conventional cell culture, the two reports illustrate the power of proteomic analysis as a hypothesis-generating tool that can identify novel pathways associated with important pathologies. These studies also remind the reader that a primary or dominant defect in extracellular matrix can have enduring effects, possibly through selection or epigenetic mechanisms, to alter many other aspects of cell behavior.

Acknowledgments

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Literature Cited

1. Dumit VI, Kuttner V, Kappler J, et al. Altered MCM Protein Levels and Autophagic Flux in Aged and Systemic Sclerosis Dermal Fibroblasts. J Invest Dermatol. 2014
2. Kuttner V, Mack C, Gretzmeier C, et al. Loss of Collagen VII is Associated with Reduced Transglutaminase 2 Abundance and Activity. J Invest Dermatol. 2014
3. Fine JD, Bruckner-Tuderman L, Eady RA, et al. Inherited epidermolysis bullosa: Updated recommendations on diagnosis and classification. J Am Acad Dermatol. 2014
4. Itahana K, Campisi J, Dimri GP. Methods to detect biomarkers of cellular senescence: the senescence-associated beta-galactosidase assay. Methods Mol Biol. 2007; 371:21–31. [PubMed: 17634571]
5. Cipriani P, Di Benedetto P, Liakouli V, et al. Mesenchymal stem cells (MSCs) from scleroderma patients (SSc) preserve their immunomodulatory properties although senescent and normally induce

T regulatory cells (Tregs) with a functional phenotype: implications for cellular-based therapy. Clin Exp Immunol. 2013; 173:195–206. [PubMed: 23607751]

6. Green DR, Levine B. To be or not to be? How selective autophagy and cell death govern cell fate. Cell. 2014; 157:65–75. [PubMed: 24679527]
7. Yang J, Zheng Z, Yan X, et al. Integration of autophagy and anoikis resistance in solid tumors. Anat Rec. 2013; 296:1501–1508.
8. Jun JI, Lau LF. Cellular senescence controls fibrosis in wound healing. Aging. 2010; 2:627–631. [PubMed: 20930261]
9. Tchkonia T, Zhu Y, van Deursen J, et al. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest. 2013; 123:966–972. [PubMed: 23454759]
10. Maier AB, Westendorp RG. Relation between replicative senescence of human fibroblasts and life history characteristics. Ageing research reviews. 2009; 8:237–243. [PubMed: 19491042]