**ABSTRACT:**

Several markers of senescent cells (SCs) identified under in vitro conditions, especially p16(Ink4a) and elevated beta-galactosidase activity (β-gal), are commonly used to detect SCs in vivo. The accuracy of this detection depends on the exclusivity of these markers. We recently reported the presence of p16(Ink4a)- and β-gal-positive macrophages (Mψ) from two sources: appearing naturally in adipose tissue of chronologically aged mice and elicited in young animals following injection of SCs embedded in alginate (LCAB model), a protective polymer which prevents rapid eradication by innate immunity [1] Hall et al., 2016, Aging 8: 1294-1315). We assessed the behavior of these markers in Mψ undergoing reversible reprogramming in response to physiological immunomodulatory stimuli. We found that M1-inducing factors [LPS, IFN-α, Poly(I:C)] caused a decrease and M2-inducing cytokines (IL-4, IL-13) increased p16(Ink4a) expression. All these stimuli modulated p16(Ink4a) in a reversible and rapid manner. In contrast to mesenchymal SCs, in Mψ the appearance of neither senescence-associated (SA)-β-gal activity (SA-β-gal) nor the accumulation of p16(Ink4a) were Mψ-specific. Consistently, agents that regulate p16(Ink4a) in Mψ reprogrammed in response to physiological immunomodulatory stimuli. We characterized the expression of p16(Ink4a) and SA-β-gal in normal tissues of aged mice. Here, we explore the role of p16(Ink4a) and SA-β-gal markers in Mψ reprogramming in response to physiological immunomodulatory stimuli. Further, roles of other senescence-associated markers in normal tissues of aged mice demonstrated reduction of both markers when subjected to senescence-inducing stimuli.

**RESULTS:**

To further characterize p16(Ink4a)- and β-gal-positive Mψ, we focused on those elicited in LCAB model

**CONCLUSIONS:**

We characterized the expression p16(Ink4a) and β-gal in Mψ using previously developed models: elicited in response to live cell-containing alginate beads (LCAB model), and naturally-occurring adipose tissue from aged mice.

- p16(Ink4a)- and β-gal-positive Mψ maintained plasticity of M1/M2 polarization phenotypes.
- Expression of p16(Ink4a) was associated with M2-like polarization: 1) LCAB-elicited Mψ were M2-like (Arg1hi iNoslo), 2) M2-inducing cytokines (IL-4 and IL-13 upregulate p16(Ink4a)), and 3) M1-polarizing agents, including Polycl(C) and IFN-α, rapidly decreased p16(Ink4a).
- β-gal and p16(Ink4a) were differentially regulated; however, LPS was shown to decrease both p16(Ink4a) and β-gal expression.
- TLR3 agonist Polycl(C) decreased p16(Ink4a) expression in LCAB macrophages in vivo. Treatment of aged mice revealed similar results in adipose tissue, suggesting that age-respose Mψ are also responsive to immunomodulatory stimuli.
- Immunomodulatory regulation of p16(Ink4a) and β-gal in Mψ, and their independence from p53 function, implies distinct signaling pathways regulating the expression (and potentially, function) of these proteins compared to senescence of mesenchymal SCs. Our research suggests that part of systemic aging-related effects previously associated with SCs could be attributed to non-senescent p16(Ink4a)/β-gal-positive Mψ accumulated in tissues of aged animals.

**REFERENCES:**

