LOSS OF SPARC IN P53-NULL ASTROCYTES PROMOTES MACROPHAGE ACTIVATION AND PHAGOCYTOSIS RESULTING IN DECREASED TUMOR SIZE AND TUMOR CELL SURVIVAL

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BACKGROUND: Both enhanced SPARC expression and the loss of p53 tumor suppressor gene are changes that occur early in glioma development. Both SPARC and p53 regulate glioma cell survival by inverse effects on apoptotic signaling. Therefore, during glioma formation, the upregulation of SPARC may cooperate with the loss of p53 to enhance cell survival and inhibit apoptosis. This study determined whether the loss of Sparc in astrocytes that are null for p53 would result in reduced cell survival and tumor formation and increased tumor immunogenicity in an in vivo xenograft brain tumor model. METHODS: p53-null and Sparc-null mice were bred to obtain heterozygotes. These were crossed to obtain p53-null/Sparc-null neonates, which served as the source of p53-null/Sparc-null astrocytes for comparison to genetically-matched, control p53-null/Sparc-WT astrocytes. In vitro, Sparc loss was assessed using colony formation in soft agar, proliferation assay, flow cytometry, and Annexin V analysis. Orthotopic xenograft implantation was used to assess Sparc loss on tumor growth, proliferation, collagen deposition, and macrophage/microglia recruitment. RESULTS: In vitro, Sparc loss in p53-null astrocytes resulted in increased cell proliferation (2.7-fold; p < 0.01), but decreased cell survival (WT = 7.2% vs. null = 18.56% apoptotic cells) and a longer cell cycle recovery (2 days) after serum-withdrawal. Furthermore, Sparc loss suppressed growth in soft agar. Histomorphological analysis of CD68 and PAS + diastase staining of tumor sections demonstrated that Sparc loss promoted macrophage/microglial activation (day 7 = 1.35-fold and day 50 = 1.93-fold; p < 0.001) and phagocytic morphology, respectively. At 7 days, Sparc-null tumors had decreased tumor cell survival, proliferation (MIB-1 for WT = 7.1% vs. null = 0.1%; p = 0.0345), and reduced tumor size (WT = 0.839 mm² vs. null = 0.199 mm²; p = 0.0091). In addition, Sparc loss altered collagen deposition (WT = collagen bundles vs. null = long fiber structure). CONCLUSIONS: Our results indicate that Sparc loss significantly reduced the ability of p53-null astrocytes to recover from stress in vitro, and to survive and proliferate in vivo. This inability to grow in vivo was associated with an increase in macrophage/microglial activation and phagocytosis. Therefore, we conclude that the loss of p53 in the early stages of glioma formation may cooperate with the induction of SPARC to potentiate cancer cell survival and escape from immune surveillance. We propose that in the background of p53 loss/mutation, therapeutically suppressing SPARC alone, or in combination with other therapies, should promote the ability of patient’s immune system to eradicate the tumor. SECONDARY CATEGORY: Immunobiology & Immunotherapy.

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