Exosomes and Macrophage Polarization in Lung Cancer

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Purpose: Lung Cancer is the leading cause of cancer related death among men and women. M2 macrophages are known suppressors of host anti-tumor immune functions and promote primary growth and relapse of treatment-resistant tumors. Recent evidence suggests that tumor cells release exosomes that promote immunesuppression. It was hypothesized that macrophages take up lung cancer cell derived exosomes and then polarize into M2 phenotypes and promote immunosuppression. Methods: Exosomes were isolated from cell culture media of human lung carcinoma cells A549 using differential centrifugation and stained with PKH26. Exosomes were then co-cultured with Thp0 cellsfor 24 and 72 hours. Thp0 cells from the control and experimental groups were then stained with anti-CD64, CD11b, CD163 and CD206 antibodies to identify M2 macrophages by flow cytometry and ImageStream analyses. Data were analyzed using FlowJo and Graph Pad Prizm. Significant differences were determined by One Way ANOVA analyses and Tukey multiple comparison test. Results: In flow cytometry, macrophages were gated using CD11b. The PKH26+ CD11b+ cells were then gated as exosome+ and M2 macrophages were identified using CD163 and CD206. ImageStream Flow Cytometry analyses confirmed that PKH26+ macrophages take up exosomes. Flow Cytometry demonstrated that exosome uptake polarized macrophages to M2 phenotype, as the addition of exosomes significantly increased the M2 macrophages from .204% (no exosome) to 1.06% (with exosomes). Conclusion: The effects of A549-derived exosomes on macrophages demonstrate its ability to promote M2 polarization. This study suggests tumor cells can secrete exosomes that can promote tumor growth and immune suppression.

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