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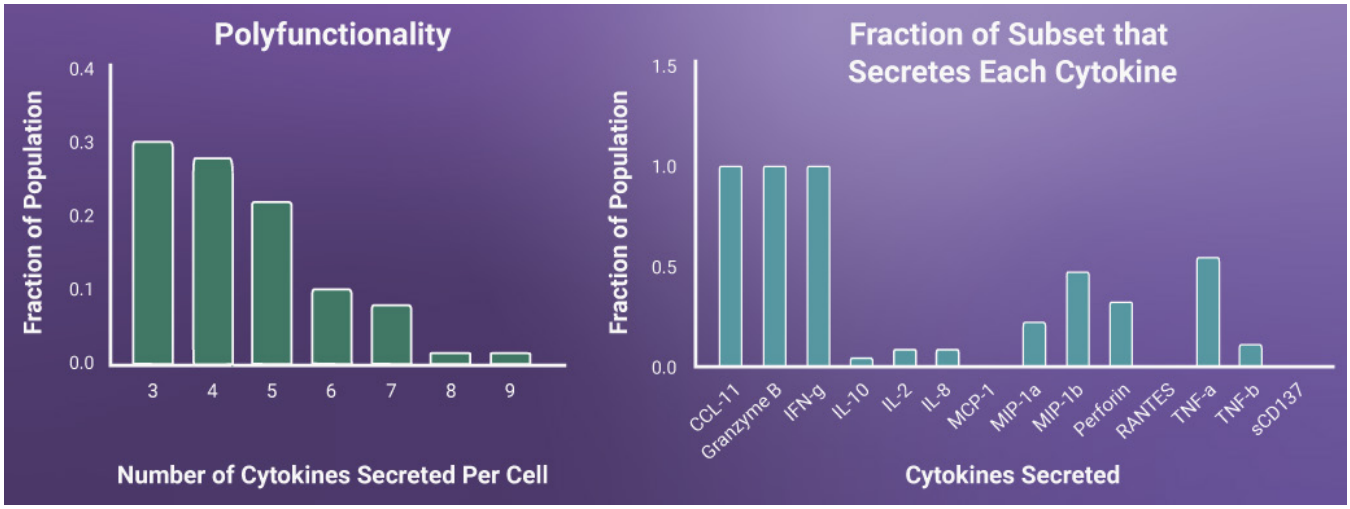
Mesenchymal stromal cell delivery of oncolytic immunotherapy improves CAR-T cell anti-tumor activity

The tumor microenvironment (TME) promotes tumor growth and metastasis while suppressing anti-cancer immune responses. This has proven to be a barrier to adoptive cell therapy. Oncolytic adenoviruses (OAds) can disrupt the TME, thereby restoring and improving immune cell function. However, delivering OAds to solid tumors is challenging. Researchers from Baylor College of Medicine took a creative approach to this problem. In a recent article published in *Molecular Therapy*, researchers outlined how mesenchymal stromal cells (MSCs) were used to directly deliver two engineered anti-cancer adenoviruses to tumor sites. Together with CAR-T cell therapy, this approach disrupted the TME, suppressed tumor growth, and encouraged T cell activity.

Overcoming natural barriers

The TME is a complex system shaped by the interplay between cancer cells, tumor-adjacent stromal cells, aberrant vasculature, and extracellular matrix. Ultimately, the TME physically impedes immune cell infiltration and produces immune-inhibitory paracrine agents, including cytokines and chemokines. The complexity and heterogeneity of this system means that narrow approaches targeting one specific effector are unlikely to succeed. Oncolytic virotherapy attacks the TME in multiple ways, disrupting and manipulating the TME to create a more immunostimulatory setting. Viruses can directly kill cancer cells, elicit anti-cancer immune responses by activating bystander T cells, and can serve as delivery vectors for genes that encode immune promoting agents, such as granulocyte-macrophage colony-stimulating factor. However, innate or adaptive immune responses often eliminate viruses before they reach the tumor site.

In response to this problem, the researchers simultaneously infected MSCs with two viruses: a cytotoxic OAd and a helper-dependent adenovirus (HDAd), thus creating a combinatorial Ad vector (CAd). OAds can infect and lyse cancer cells directly. In contrast, HDAds have the cargo capacity to express a broad array of immuno-



Mesenchymal stromal cell delivery of oncolytic immunotherapy increased CAR-T cell polyfunctionality and efficacy.

modulatory transgenes and can be engineered to infect non-malignant cells within the TME, thereby inducing sustained immunostimulatory cytokine production.

CAd-MSCs stimulate CAR-T cell cytokine production in vitro

After establishing that CAds infect MSCs, researchers created HDAds encoding transgenes for interleukin-12 (IL-12) and programmed death-ligand 1 (PD-L1) blocking antibody, thus creating CAd12_PD-L1 MSCs. The team placed these cells alongside clinically validated tumor-directed HER.2 CAR-T cells and used functional single-cell analysis to find that the presence of CAd-infected MSCs resulted in enhanced CAR-T cell anti-tumor activity *in vitro*. Specifically, CAR-T cells showed increased cytokine secretion frequency when cocultured with CAd-MSCs, compared to either no MSCs or uninfected MSCs. Similarly, elevated proportions of both CD4+ and CD8+ T cells secreted cytokines upon antigen-expressing tumor cell stimulation when CAd-MSCs were present. Finally, the proportion of polyfunctional T cells among cytokine-secreting T cells was significantly greater in the presence of CAd-MSCs. In previous studies, polyfunctionality has been associated with anti-tumor activity and has predicted clinical outcomes.

Delving deeper into this polyfunctionality, using the bioinformatics software integrated with the functional single-cell proteomics platform used previously, the team found a subset of CD4+ HER.2 CAR-T cells co-cultured with CAd-MSCs that secreted up to nine cytokines per cell, including the pro-inflammatory cytokine IFN-γ, the pro-apoptotic protease granzyme B, and the pro-chemotaxis chemokine CCL11. This presented a contrast to CD4+ CAR-T cells that were co-cultured with uninfected MSCs, which primarily secreted IL-8. They also found that CD8+ HER.2 CAR-T cells that had been co-cultured with CAd-MSCs secreted 2-7 cytokines per cell, while CD8+ CAR-T cells that were not co-cultured with MSCs, but were stimulated by tumor cells, secreted 4-6 cytokines per cell. Both CD8+ populations secreted IFN-γ, granzyme B, TNF-α, and perforin, but CAR-T cells co-cultured with CAd-MSCs did not produce MIP1α and MIP1β, suggesting a more cytolytic phenotype. These results indicate that CAd-MSCs had a more pronounced effect on CD4+ T cells, eliciting increased production of pro-inflammatory mediators by these cells upon exposure to tumor cells.

CAd-MSCs promote CAR-T cell-mediated tumor reduction in vivo

The study next examined the effects of CAd12_PD-L1 MSCs on HER.2 CAR-T cell anti-tumor activity in more physiologically applicable models. First, they introduced CAd-MSCs to 3D tumor spheroids and tracked MSC integration and viral infection spread using dyes. Not only was viral spread profoundly visible, but spheroids treated with CAd-MSCs and CAR-T cells showed considerably elevated tumor cell cytotoxicity compared to those treated with uninfected MSCs or CAR-T cells alone. Likewise, the introduction of both CAd-MSCs and CAR-T cells markedly reduced tumor growth in two murine non-small-cell lung carcinoma xenograft models, one using A549 cells and the other using H1650 cells. In the A549 model, CAR-T treatment alone could temporarily reduce tumor growth, but only the combination of CAd-MSCs and CAR-T cells prevented tumor growth throughout the 22-day examination period. Moreover, only animals that received both CAd-MSCs and CAR-T cells withstood a second challenge with A549 cells. Accordingly, T cells in animals receiving both CAd-MSCs and CAR-T cells showed fewer indicators of exhaustion following this second challenge. Performance was even better in the H1650 model, with combination treatment resulting in increased T cell infiltration and complete tumor clearance within two weeks of HER.2 CAR-T cell infusion.

Highlighting the importance of a multi-pronged approach for superior therapeutic efficacy

The study showed that a combination of immunostimulatory CAd-MSCs and CAR-T cells improved T cell effector function, endurance, and infiltration into bulky tumors. They also determined that by delivering the transgenes IL-12 and anti-PD-L1 to tumor sites, CAd-MSCs exert this effect by promoting T cell polyfunctionality. These insights were uniquely revealed using a single-cell functional proteomic platform, which also identified correlations with *in vivo* biology and anti-tumor response in mouse models. This study highlights how a multi-pronged approach can offset and overcome monotherapy-specific weaknesses to yield better treatment efficacy.

REFERENCE

M. McKenna et al., "Mesenchymal stromal cell delivery of oncolytic immunotherapy improves CAR-T cell antitumor activity," *Mol Ther*, S1525-0016(21)00071-X, 2021.