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Tumor-derived exosomes modulate T cell function through transfer of RNA

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Recent successes in the clinic with cancer immunotherapies, such as immune checkpoint blockade and adoptive cellular transfer, have drawn significant attention towards the complex interplay between a developing tumor and the immune system. Both endogenous anti-tumor immune responses and the success of immune-based therapies depend upon cells that directly target and eliminate tumor cells, such as natural killer cells and cytotoxic T lymphocytes (CTLs). Through the process of immune

editing, cancer cells can develop means to evade the immune system, including the upregulation of immune checkpoints, such at PD-L1, the down regulation of machinery required for tumor antigen presentation, promotion of immunosuppressive cells, such as T regulatory cells, and the production of immunosuppressive cytokines [1, 2]. An emerging area of research is the impact of tumor-derived extracellular vesicles (exosomes) on modifying the tumor microenvironment. The secretion of exosomes has been linked to the formation of a 'premetastatic niche' and modulation of immune cell function [3-5]. More specifically, it has been recently shown that protein and mRNA loaded tumor-derived exosomes can directly impact CTL function [6].

In this issue of *The FEBS Journal*, Bland *et al.* have built upon their previous work [6] and explored the mechanism by which tumor-derived exosomes alter CTL function [7]. The authors initially determined that B16F0-GFP melanoma cells are capable of producing exosomes, characterized by expression of protein biomarkers such as CD63 and Hsp70, that are able to transfer GFP into a CTL cell line, CTLL-2. In order to investigate the biological significance of this transfer, Bland *et al.* investigated the mRNA and miRNA 'payload' of the exosomes by microarray. This analysis revealed that ~30% of the miRNAs were overrepresented in exosomes as compared to the parental B16F0 cells. A number of miRNAs and mRNAs identified as being highly represented were subsequently validated by qRT-PCR. Interestingly, target pathway analysis of the miRNAs upregulated in exosomes revealed some immunological pathways as potential targets, including interferon signaling [7].

Exposure of CTLL-2 cells to B16F0-derived exosomes led to significant changes in the transcriptome of CTLL-2 target cells within a short time frame. Notably, the authors were able to show that 4 of the top 20 mRNAs expressed in B16F0 exosomes (Wsb2, Fam168b, Cmtm4 and Ptpn14) were upregulated in CTLL-2 cells within 30 minutes. Two subsequent clusters of genes were identified as being regulated at later time points, including one set that was upregulated at 4 hours following exosome treatment and contained several gene targets involved with cellular metabolism. Functional characterization of exosome-exposed CTLL-2 cells showed that these cells had increased mitochondrial respiratory activity, as measured by Seahorse assay, as well as increased IFNγ production. Given the well documented link between metabolism and T cell fate and function within the tumor microenvironment [8], this result is striking as it may suggest that tumor cells can utilize exosomes to modify CTL function through alteration of cellular metabolism. Taken together these results suggest a model in which cancer-cell-derived exosomes are able to transfer mRNA and miRNA to CTLs and reprogram their functional activity (**Figure 1**).

This study raises interesting questions concerning the importance of mRNA and/or miRNA transferred to immune cells via cancer-derived exosomes. Interestingly, despite the transfer of a number of miRNAs from B16F0 cells, Bland *et al.* detected no observable downregulation of target mRNA within CTLL-2 cells. This is somewhat surprising given the significant enrichment of miRNAs, such as miR-709 and miR-2137, in exosomes when compared to parental cells. However, given that miRNAs can directly alter translation without affecting transcript abundance [9, 10], it remains to be seen whether these miRNAs impact cellular functions through a direct effect on translation rates. Consequently, a further investigation of the proteome in exosome-treated cells may reveal a much broader range of effects on CTL function. Such an investigation may also confirm that exosome delivered mRNAs are in fact translated to proteins within target cells. This is of particular interest for the identified gene targets involved in Notch-1 signaling, given the role of Notch signaling in upregulation of cytokines and cytotoxic machinery, both important facets of the CTL response [11]. Moreover Bland *et al.* identified CMTM4 mRNA as being upregulated in exosome-exposed CTLL-2 cells, this is potentially significant given its recently described role in PD-L1 trafficking [12].

These findings by Bland *et al.* now warrant further investigation into the impact of tumor-derived exosomes on a broad range of murine and human primary cells. While CTLL-2 cells provide an interesting proof of concept, it is likely that the translational/transcriptional plasticity of primary CTLs is significantly divergent from that of the immortalized CTLL-2 cell line focused on in the current study. This may in turn affect the impact exosomal miRNA/mRNAs has on CTL function. Moreover, as exosome delivered miRNA/mRNA were found to alter metabolism rather than a specific CTL function (i.e. cytotoxicity), it is likely that tumor-derived exosomes could impact the function of a broad range of immune cell types, as has been previously described [13]. Such an investigation may reveal a broader understanding of how tumor-derived exosomes help a developing cancer suppress and evade the immune response.

In conclusion, the paper of Bland *et al.* has demonstrated the novel finding that mRNA/miRNA loaded tumor-derived exosomes have the capacity to alter the metabolic function of CTLs. Further investigation of this phenomenon in a broader range of cells and *in vivo* has the potential to delineate an additional mechanism by which tumor cells can alter and evade the immune system for their survival.

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Figure Legend

Transfer of exosome-derived mRNA and miRNAs impacts CTL metabolism

mRNA- and miRNA-loaded exosomes are released from cancer cells into the tumor microenvironment (**A**) and delivered to CTLs (**B**) leading to modulation of their metabolism and cytokine production (**C**).