Plasma and exosome proteomic profiling for prediction of immunotherapy response and toxicity

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Immune checkpoint blockade (ICB) has revolutionized the treatment of many solid tumors, including metastatic melanoma. Despite recent successes, many patients fail to respond or are overcome by severe toxicities that limit further treatment. To date, there are no non-invasive predictors of response and toxicity that can guide treatment decisions. In this work, we perform whole plasma and exosome proteomic profiling to construct a predictive model of immunotherapy response and toxicity, and to glean further biologic insight into the mechanisms underlying resistance to ICB. Whole plasma was analyzed in a cohort of 150 melanoma patients receiving anti-PD1 antibodies (MGH IRB #11-181) at baseline, and on-treatment at 6 week and 6 month time-points. Exosomes were analyzed in 15 of these patients for all time-points. Proteomic analysis was performed using an innovative multiplex proximity extension assay that enabled detection of more than 1000 proteins simultaneously. A linear mixed model with maximum likelihood estimation for model parameters was used to analyze differences between patient groups, and significant differences were determined after Benjamini and Hochberg multiple hypothesis correction. Between plasma baseline and on-treatment time-points, 67 differentially expressed proteins were identified including markers of inflammation such as PD1, CXCL9, CXCL10, CXCL11, IL10, CCL3 and TNFR2. Exosome samples had a distinct protein signature over the treatment period compared to plasma, including differential expression of CXCL16, CCL18, CCL20, and IL6, among others. 41 proteins were differentially expressed in plasma between ICB responders and non-responders including several inflammatory proteins such as CD28, TNFβ, MCSFRa and IL8, and others implicated in melanoma resistance, such as MIA and ERBB2. Again, exosome revealed a distinct protein signature between responders and non-responders compared to plasma consisting of CXCL9, CXCL13, CXCL16, CCL19, CD8a, GZMA and CD5 expression. Whereas plasma proteins reflected a myeloid signature, exosome proteins reflected a lymphoid signature, suggesting that the two compartments may capture elements of different immune processes. Integrating data from both plasma and exosome proteomics, we applied machine learning tools to build a predictor of response. Further analysis to look for predictors of toxicity is currently underway. Overall, our work suggests that plasma and exosome protein signatures are distinct and may reflect unique immunological processes. Proteomic analysis of these compartments may be an effective way for non-invasive liquid biopsy to predict ICB response.