Variation in cell signaling protein expression may introduce sampling bias in primary epithelial ovarian cancer.

Abstract:
Although the expression of cell signaling proteins is used as prognostic and predictive biomarker, variability of protein levels within tumors is not well studied. We assessed intratumoral heterogeneity of protein expression within primary ovarian cancer. Full-length proteins were extracted from 88 formalin-fixed and paraffin-embedded tissue samples of 13 primary high-grade serous ovarian carcinomas with 5-9 samples each. In addition, 14 samples of normal fallopian tube epithelium served as reference. Quantitative reverse phase protein arrays were used to analyze the expression of 36 cell signaling proteins including HER2, EGFR, PI3K/Akt, and angiogenic pathways as well as 15 activated (phosphorylated) proteins. We found considerable intratumoral heterogeneity in the expression of proteins with a mean coefficient of variation of 25% (range 17-53%). The extent of intratumoral heterogeneity differed between proteins (p<0.005). Interestingly, there were no significant differences in the extent of heterogeneity between phosphorylated and non-phosphorylated proteins. In comparison, we assessed the variation of protein levels amongst tumors from different patients, which revealed a similar mean coefficient of variation of 21% (range 12-48%). Based on hierarchical clustering, samples from the same patient...
clustered more closely together compared to samples from different patients. However, a clear separation of tumor versus normal tissue by clustering was only achieved when mean expression values of all individual samples per tumor were analyzed. While differential expression of some proteins was detected independently of the sampling method used, the majority of proteins only demonstrated differential expression when mean expression values of multiple samples per tumor were analyzed. Our data indicate that assessment of established and novel cell signaling proteins as diagnostic or prognostic markers may require sampling of serous ovarian cancers at several distinct locations to avoid sampling bias.