Protein phosphorylation plays a significant role in various biological processes. Cancer progression is also regulated by alteration of protein phosphorylation. In this study, we quantitatively analyzed the alteration of protein phosphorylation in cancer tissues to identify novel phosphorylation signals involved in colorectal cancer progression, especially in cancer metastasis. We performed mass spectrometry-based quantitative phosphoproteomic analysis using surgically extracted colorectal cancer tissues. Tryptically digested phosphopeptides were purified by immobilized metal-affinity chromatography (IMAC) and subsequently labeled with isobaric tags for relative quantification (iTRAQ). Phosphopeptide identification and quantification were performed by LTQ Orbitrap Velos. Possible kinase-substrate relationships were predicted by NetworKIN algorithm. A total of 10477 distinct phosphopeptides were identified and hundreds of phosphopeptides were differentially expressed between metastatic and nonmetastatic cancer tissues. Prediction with NetworKIN suggested that substrates of specific kinase groups were over- or under-phosphorylated in metastasic cancer tissue, which are potential metastatic biomarker for diagnosis and treatment of colorectal cancer.

**Keywords**: Phosphoproteomics, Cancer