Phosphoproteome and Protein Kinase Activity in Fresh-Frozen Colorectal Cancer Tissue Obtained from Patients.

Xiao Wang*

Department of Medical Oncology, Radboud University Medical Center, Geert Grooteplein 10, The Netherland

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Abstract

Kinase activity profiling in tumour samples by peptide microarrays and mass spectrometry-based phosphoproteomics may influence the selection of protein kinase inhibitors in cancer patients due to their ability to detect aberrant cellular signaling in connection to biological function. Variable tissue handling processes in clinical practise can affect protein phosphorylation state and kinase activity, making biomarker finding more difficult. Using peptide microarrays and mass spectrometry-based phosphoproteomics, the influence of cold ischemia time (CIT) on the stability of kinase activity and protein phosphorylation status in fresh-frozen clinical tissue samples was investigated. Five patients' biopsies of colorectal cancer resection specimens were taken and snap frozen immediately after surgery and at intervals between CIT. A peptide microarray was used to profile kinase activity in all samples. At several time points, MS-based global phosphoproteomics was done in malignancies from patients. Changes in kinase activity and phosphoproteome as a result of CIT were studied using statistical and cluster analysis. The bulk of the phosphoproteome and protein kinase activity in colorectal cancer resection tissue is stable up to minutes after CIT and reflects tumour features. However, with increased CIT, specific alterations in kinase activity were detected. To limit changes in kinase activity during CIT, strict tissue collection protocols are recommended.

Keywords: Protein Kinase, Phosphoproteome, CIT, PKIs, Fresh-Frozen, Patients, Cancer Tissue.

Introduction

Protein kinases play an important role in biological activities like cell motility, proliferation, differentiation, and survival. Changes in protein kinase activity can disrupt normal cellular signalling, causing cells to become cancerous. Cancer cell proliferation can be slowed by inhibiting the function of unregulated kinases. Protein kinase inhibitors (PKIs) have become therapeutically available for the treatment of many cancer types in recent decades [1]. When it comes to molecular profiling tactics for predicting the efficacy of targeted medicines in specific patients, procedures that take into account the mechanisms of action and level of activity are suitable. In essence, all currently available targeted medicines target signalling molecules rather than genes. Gene expression studies and protein abundance may be informative, but analysing networks that underpin cancer development, progression, and therapy resistance at both a personal and system-wide level would be a superior alternative [2]. Peptide microarray-based kinase activity profiling and phosphoproteomics are two such approaches that provide unique information on the complicated signalling pathways changed in cancer cells. They may also aid in the development of new biomarkers and therapy targets [3].

Still, one of the obstacles in applying such technologies is the need for fresh frozen tumour tissue samples, as formalin-fixed paraffin-embedded (FFPE) material is not acceptable due to kinase activity and protein phosphostatus being affected by the fixation technique. The freezing method and storage of tumour tissue may impact cellular signalling and protein phosphorylation levels in clinical sample handling and tissue processing. Kinase activity and protein phosphostatus can be affected by the interval between surgical tumour removal and freezing, referred to as cold ischemia time (CIT). The finding of biomarkers utilising fresh frozen material could be aided by CIT [4].

CIT's influence on protein phosphorylation has been documented in previous mass spectrometry investigations to range from steady to unanticipated changes in phosphosites. Using peptide microarrays, the influence of CIT on kinase activity patterns has never been explored before. Using colorectal cancer (CRC) tissue samples obtained in a clinical environment, we study the impact of cold ischemia on cellular signalling activity [5].

Conclusion

As a result, quick changes were overlooked. Furthermore, pTyr sites are thought to be more prone to ischemia than

pSer/pThr sites, which were mostly discovered in this work due to the abundance of serine/threonine phosphosites in the phosphoproteome. In conclusion, this work reveals that the bulk of the phosphoproteome, as well as protein kinase activity, remains stable in colorectal cancer resection tissue. However, kinase activity and protein phosphorylation status may alter throughout CIT, and kinase activity and phosphoproteome changes may vary by tumour sample. The phosphoproteome and kinase activity still represent tumour features, hence these alterations during CIT do not dramatically obfuscate tumour traits. Although heterogeneity and tumor-specific responses to ischemia may impact kinase activity patterns, this only accounts for a small percentage of phosphopeptides when assessed following CIT. Despite these minor discrepancies, standardised tissue collecting protocols are recommended to reduce variability in phosphoproteome and protein kinase activity measurements during CIT.

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***Correspondence to:**

Xiao Wang
Department of Medical Oncology,
Radboud University Medical Center,
Geert Grooteplein 10, The Netherland
E-mail: Xiaowang@edu.cn

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