Deparaffinization and Proteome Analysis from FFPE Tissues of Colon Cancer

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INTRODUCTION: The colorectal cancer (CRC) is one of the most common in Brazil. Its diagnosis is difficult and invasive to patients. Thus, early detection of CRC by noninvasive methods is needed. The use of tissues fixed in formaldehyde and embedded in paraffin (FFPE) represents an immeasurable resource for the discovery of these biomarkers related to CRC. However, the fixation with formaldehyde results in crosslinking of proteins which limits an efficient protein extraction. The aim of this study is to identify proteins related to CRC using 2D-GE and deparaffinized tissues.

MATERIAL AND METHODS: Paraffin blocks were treated with different organic solvents and immerse in ethanol. The proteins were obtained in a buffer containing 2\% SDS or CHAPS 4\% and subject to 100°C for 20 min, 80°C for 2 h and then clarified for 15 min at 12000×g at 4°C. The concentration of proteins was determined by 2-DQuant kit. The protein fraction was separated in the first dimension by isoelectric focusing and in the second dimension by SDS-PAGE 12.5\% and the gel was stained with comassie blue.

RESULTS AND DISCUSSION: Based on the results of total protein quantification, we observed that the protein concentration when deparaffinized with heptane (4.73 µg.µL\textsuperscript{-1}) was two time greater than from the other solvents (xylene 2.50 µg.µL\textsuperscript{-1}, hexane 2.49 µg.µL\textsuperscript{-1}, octane 2.24 µg.µL\textsuperscript{-1}). Furthermore, the samples extracted with SDS in the buffer showed greater yield than with CHAPS. However, when those samples with surfactants were subjected to 2D-GE we observed a diffuse band of proteins. One hypothesis is that the addition of SDS prevents sample focusing.

CONCLUSION: Thus far, the analysis of proteins by 2D-GE showed not effective when FFPE samples were extracted with SDS with heat. An alternative method to 2D-GE separation is underway using isoelectric focusing fractionation off-gel and analysis of peptides by LC-MS.

Keywords: colorectal cancer, FFPE tissues, 2D-GE.
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