European Organisation for Research and Treatment of Cancer (EORTC) Pathobiology Group standard operating procedure for the preparation of human tumour tissue extracts suited for the quantitative analysis of tissue-associated biomarkers.

Abstract:

With the new concept of 'individualized treatment and targeted therapies', tumour tissue-associated biomarkers have been given a new role in selection of cancer patients for treatment and in cancer patient management. Tumour biomarkers can give support to cancer patient stratification and risk assessment, treatment response identification, or to identifying those patients who are expected to respond to certain anticancer drugs. As the field of tumour-associated biomarkers has expanded rapidly over the last years, it has become increasingly apparent that a strong need exists to establish guidelines on how to easily disintegrate the tumour tissue for assessment of the presence of tumour tissue-associated biomarkers. Several mechanical tissue (cell) disruption techniques exist, ranging from bead mill homogenisation and freeze-fracturing through to blade or pestle-type homogenisation, to grinding and ultrasonics. Still, only a few directives have been given on how fresh-frozen tumour tissues should be processed for the extraction and determination of tumour biomarkers. The PathoBiology Group
of the European Organisation for Research and Treatment of Cancer therefore has devised a standard operating procedure for the standardised preparation of human tumour tissue extracts which is designed for the quantitative analysis of tumour tissue-associated biomarkers. The easy to follow technical steps involved require 50-300 mg of deep-frozen cancer tissue placed into small size (1.2 ml) cryogenic tubes. These are placed into the shaking flask of a Mikro-Dismembrator S machine (bead mill) to pulverise the tumour tissue in the capped tubes in the deep-frozen state by use of a stainless steel ball, all within 30 s of exposure. RNA is isolated from the pulverised tissue following standard procedures. Proteins are extracted from the still frozen pulverised tissue by addition of Tris-buffered saline to obtain the cytosol fraction of the tumour or by the Tris buffer supplemented with the non-ionic detergent Triton X-100, and, after high-speed centrifugation, are found in the tissue supernatant. The resulting tissue cell debris sediment is a rich source of genomic DNA.