Characterization of Nipple Aspirate Fluid (NAF) by Two-Dimensional Gel Electrophoresis

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Worldwide, breast cancer is the main cause of cancer deaths in women. According to the Brazilian National Cancer Institute (INCA), 49,400 new cases were expected throughout the country in 2008. Most cases originate from mammary ductal cells that secrete NAF, a fluid containing proteins associated with the tumor microenvironment. The aim of this study is to use a proteomic approach to reliably compare NAF samples from tumorous and non-tumorous breasts of women with unilateral breast cancer. Average protein concentration in NAF from healthy breasts and tumorous ones (both with cancer or benign lesions) was 133.3 µg/µL ± 49.7 (n =15). On 2D-PAGE, most NAF protein spots focused between pH 4 and 7, with molecular masses ranging from 14.4 to 97 kDa. Optimum protein separation and detection were achieved after cup loading sample application and fluorescence DIGE analysis, respectively. After in-gel trypsinization and MALDI-TOF/TOF analysis, several typical plasma proteins (*e.g.* albumin, IgG, IgA, antitrypsin and tranferrin) were equally identified in NAF. Other proteins potentially associated with breast cancer could also be detected in NAF, including prolactin-induced protein, apolipoprotein-D and zinc-a2-glycoprotein. NAF was submitted to Agilent immunoaffinity chromatography (removes top-6 highest abundant proteins from human plasma) and important non-specific binding was noticed, reason why this methodology was not further used as a pre-purification strategy. After preliminary NAF characterization, we can now use optimized DIGE conditions to compare NAF from healthy and cancer patients, trying to identify biomarker candidates for the early diagnosis of breast cancer.

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