Levels of activated sugars in breast cancer cells reflect metastatic potential as well as cell density. A ^{31}P NMR spectroscopic study of cell extracts.

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INTRODUCTION

Cancer metastases are responsible for the fatal effects of many malignant tumors. At present, it appears to be clear that cell-cell recognition plays a fundamental role in the invasiveness of cancer cells and in their potential to attach to other tissues to form secondary tumors. Oligosaccharides in plasma membrane glycoproteins are important in cell-cell recognition and molecular targeting. Thus, alterations in oligosaccharide metabolism can be expected to affect cell-cell interactions and, potentially, the ability of cancer cells to disaggregate from a primary tumor, to invade surrounding tissue and to attach to a metastatic site. Previous work has shown that intracellular levels of activated sugars (= UDP-hexoses, UDP-Hex) may be correlated with tumorigenicity, malignancy and metastatic potential of cancer cells [1,2], but may also depend on growth conditions, differentiation status and antioxidant resistance [3-5]. This pilot study is aimed at gaining new insight into saccharide metabolism in metastatic vs. non-metastatic breast cancer cell lines, and at exploring the potential of ^{31}P NMR spectroscopy of UDP-Hex to detect the metastatic potential of breast cancer.

MATERIALS AND METHODS

Sample preparation Two human breast carcinoma cell lines were used: MCF-7 (non-metastatic) and MDAmb-435 (highly metastatic). Cells were grown to ca. 80% or 100% confluence, extracted with perchloric acid and prepared for ^{31}P NMR spectroscopy as previously described [6].

NMR spectroscopy ^{1}H-decoupled ^{31}P NMR spectra were obtained at 202.5 MHz on a 11.7 T Bruker AVANCE DRX500 NMR spectrometer using a broadband probe for 5-mm tubes. The acquisition time AQ was 2.025 s, corresponding to 8k data points, and the sweep width SW was 2 kHz. Spectra were acquired at 4°C over 6-10 h with a repetition time of TR = 13 s to avoid saturation effects.

Statistics Two-way ANOVA was used to determine the significance of differences in relative UDP-Hex levels between groups.

RESULTS

Two distinct UDP-Hex signal groups could be readily integrated, the β-phosphates of UDP-*N*-acetylglucosamine (UDPGlcNAc), and those of other UDP-Hex primarily consisting of UDP-*N*-acetylgalactosamine and UDP-glucose. Relative levels were determined by dividing UDP-Hexβ by NTPβ integrals, a quantitation method commonly used in in-vivo ^{31}P NMR spectroscopy [7]. All relative UDP-Hex levels varied as a function of both metastatic potential *and* cell density (Fig. 1). This was confirmed by two-way ANOVA which yielded *signifcant interaction* ($p < 0.05$) between the two factors, cell type and confluence, for total UDP-Hex (Fig. 1, top graph) and UDPGlcNAc (data not shown).

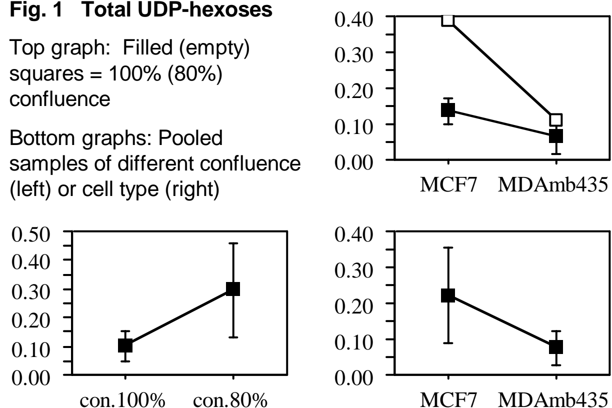

Fig. 1 Total UDP-hexoses

Top graph: Filled (empty) squares = 100% (80%) confluence

Bottom graphs: Pooled samples of different confluence (left) or cell type (right)

DISCUSSION

Increased UDPHex/NTP values for MCF7 vs. MDAmb-435 cells have been recently reported for perfused cells [8]; however, our work demonstrates that this difference becomes insignificant when cells reach very high densities in culture (ca. 100% confluence). Thus, cell density has a significant effect on the cell type dependence of UDP-Hex accumulation, and conversely, the cell density dependence of UDP-Hex accumulation is significantly affected by the cell type. This interdependence of two (or more) factors, known as statistical interaction, results in large standard deviations for UDP-Hex if factors are not separated (Fig. 1, bottom graphs). Our results suggest that the usefulness of future in-vivo assessment of the metastatic potential of breast cancer by ^{31}P NMR spectroscopy of UDP-Hex, will most likely depend on the growth characteristics of the tumor under investigation.

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