Combining data from HR MAS MR spectroscopy and gene expression can refine the subclassification of breast cancer

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Background

Transcriptional portraits of breast cancer have led to the identification of five different subgroups of patients with significantly different outcomes (Perou et al. 2000, Sørlie et al. 2001). The luminal A subgroup is the largest and is associated with positive ER status and relatively good prognosis. However, a certain fraction of patients with this type of cancer have a poor outcome. It is of interest to identify molecular differences within the luminal A group which could ultimately predict those that should receive a more aggressive treatment and those that should not. The biochemical activity in cancer tissue is also altered and HR MAS spectral profiles of breast cancer tissue correlate to clinical findings (Bathen et al. 2006). The aim of this study is to explore whether data from HR MAS and gene expression, alone or in combination, could identify such a subgroup.

Materials and Methods

Tissue samples were collected from 51 breast cancer patients and stored in liquid nitrogen until analysis. Two samples from each patient were analysed by HR MAS. The biopsies (av. weight 20.5 mg) were cut to fit a 4 mm zirconium rotor with PBS-TSP buffer. HR MAS experiments were performed on a Bruker AVANCE DRX600 spectrometer (5 kHz spin, 4 °C) and experiments were performed as previously described (Sitter et al. 2006). After MR analysis, one of each pair of biopsies was fixed in 10% formalin and embedded in paraffin for histopathology. The relative areas of normal and neoplastic epithelial elements, necrotic tissue, fat and fibrous connective tissues were scored by a pathologist. Total RNA was extracted from the second biopsy from each patient, following the protocol for the RNeasy Mini Kit (Qiagen)). Good quality RNA (as measured by BioAnalyzer 2100, Agilent) was used in two-colour microarray experiments, using 44k Agilent Human Whole Genome Oligo Microarrays. For 11 of the samples, RNA was extracted and processed from a neighbouring tissue sample not analysed by MAS. A separate study has shown that there is no dramatic effect of the HR MAS procedure on RNA integrity or gene expression (unpublished data). Three patients were excluded from the dataset due to poor array quality. The final dataset included HR MAS spectra and expression microarray data from 48 patients. After preprocessing of the microarray and HR MAS spectroscopy data, further analysis was performed using the open source software R and Bioconductor. Analyses performed so far include hierarchical clustering, multidimensional scaling and Gene Set Analysis (Efron and Tibshirani 2006).

Results and Discussion

All samples were classified into the five intrinsic subgroups according to gene expression (Hu et al. 2006). The majority were classified as luminal A. Using an inclusion criterion of at least 10 % tumour tissue from histopathology, 29 luminal A samples were selected for further analyses. Using hierarchical clustering and multidimensional scaling, two fairly robust groups were revealed using the HR MAS spin echo spectra (Figure 1). Important metabolic differences between the two luminal A groups in these spectra are lipid signals. Preliminary results from Gene Set Analysis of the gene expression data show that the subgroup with higher lipid signals is associated with apoptosis. Lipids in cancer have earlier been associated with apoptosis (Hakumäki et al. 1999). Lipids can also represent triglycerides which may reflect the tissue composition of the samples. However, there is no significant difference in the stroma components between these two luminal A groups. The clinical value of the transcriptional differences between the two groups will be investigated in a different breast cancer gene expression microarray dataset with long term follow up.

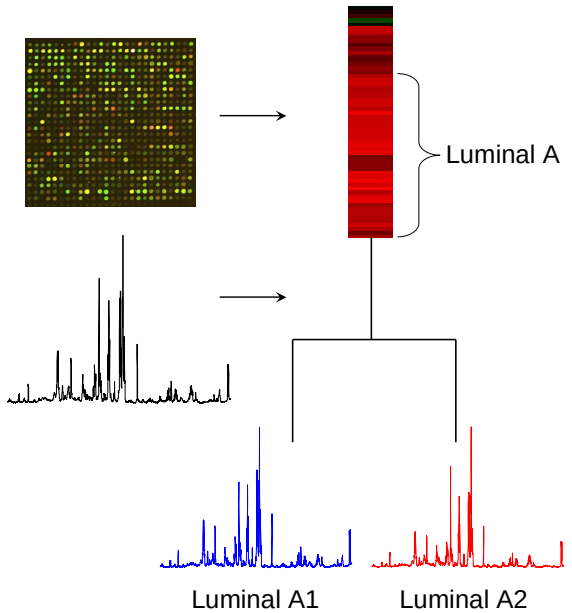

Figure 1. Combining expression microarrays and HR MAS to find two subgroups of luminal A samples**.**

Conclusion

The combination of gene expression microarrays and HR MAS spectroscopy has the potential to reveal more refined subgroups of breast cancer based on molecular properties of the tumor. Splitting the breast carcinomas classified by gene expression as luminal A into two groups based on metabolic differences, can give further biological insight into the differences in this subgroup. This could ultimately lead to a better understanding of which patients should receive a more aggressive treatment.

References

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