sheep. Only a few proteins have been identified as being part of the ESP, and most of them have never really been proved as being secreted. To cast light on the mechanism of infection and to provide information for future vaccine development, the aim of the present study was to characterize the proteins in the ESp, and to determine which proteins are immunogenic (2).

Protein expression quantification

One of the major aims of proteomics is to provide quantitative data on differential protein-expression levels, for instance in healthy and diseased states. Conventially, the proteomics approach uses protein separation by 2D-gel electrophoresis, followed by staining of the proteins. The image analysis of the 2D gels provides quantitative data on protein expression levels. More recently, mass spectrometry based methods have been introduced that can provide quantitative data on differential protein expression, mostly using stable isotope labeling. A very promising method is the so-called isotope-coded affinity-tags method. In our work we have however chosen to use metabolic labeling to introduce stable isotopes in the proteins. We have found that metabolic labeling with stable isotopes provides efficient means to quantify differential protein expression by mass spectrometry. It has the advantage over chemical-labeling methods that no derivatization is needed and that all proteins are labeled universally. Stable isotope labeling has so far been limited to lower organisms because of their ability to grow in defined media. In the present study we aimed to label the multicellular organisms *Drosophila melanogaster* (fruitfly) and *C. elegans* with ^{15}N. The method is applied to the analysis of a *C. elegans* mutant unable to generate a germline. Furthermore we show that this can be approached by 2D-gel electrophoresis and MALDI-TOF MS as well as LC-MS/MS based methods.

Literature

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Expression profiling changes treatment in breast cancer

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Microarray gene expression profiling combined with advanced bio-informatics is beginning to show its power in delineating disease entities that are otherwise indistinguishable. This refinement in tumor classification allows a more accurate prediction of outcome of disease for patients that present with the same stage of disease based on conventional clinical and histopathological criteria. Gene activities determining the biological behaviour of the tumor may indeed be more likely to reflect the aggressiveness of the tumor than general parameters like tumor size, age of the patient, or even tumor grade. Therefore, the immediate clinical consequences are that treatment schemes can be tailored based on the gene-activity patterns of the primary tumor.

We used gene expression profiling with DNA microarrays harboring 25.000 genes on 78 primary breast cancers of young lymph-node negative patients to establish a signature, predictive for a short interval to distant metastases. This 'poor prognosis' signature consists of genes involved in cell cycle, invasion and angiogenesis. The prognosis signature is superior to currently available clinical and histo-pathological prognostic factors in predicting outcome of disease (OR=18 (95%CI 3.3-94), $p<0.001$, multivariate analysis). At present we have validated our findings of this poor-prognosis profile on a large independent series of LN0 as well as LN+ (lymph-node positive) young breast-cancer patients (n=187). Preliminary analysis confirms that the profile is a strong factor in predicting outcome of disease for LN0 patients (OR=17). Furthermore, the profile is as powerful for LN+ patients (OR=12).

Nowadays, consensus guidelines in the management of breast cancer select up to 90% of lymph-node negative young breast-cancer patients for adjuvant systemic therapy (e.g., St Gallen). As 70-80% of these patients would have remained disease-free without this adjuvant treatment, these patients are 'overtreated'. Our 'poor prognosis' signature provides a novel strategy to accurately select patients who would benefit from adjuvant systemic therapy and can greatly reduce the number of patients that receive unnecessary treatment.

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