



## **Molecular Genetics of Lewis and Secretor Genes**

**Influence of Secretor and Lewis Allelotypes on Concentrations of Tumour-Associated Carbohydrate Structure Sialyl-Lewis  $\alpha$  in Serum and Urine from Bladder Cancer Patients**

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This thesis is based on studies carried out during my employment as a research fellow to professor Hans Wolf in the Department of Urology, Aarhus University Hospital, Skejby, 1995-1999. The work was done in the research laboratories of the Department of Clinical Biochemistry, Aarhus Municipal Hospital and the Molecular Diagnostic Laboratory, Department of Clinical Biochemistry, Skejby Hospital.

Fucosylated glycoconjugates belonging to the Lewis blood group system have attracted rather much scientific attention during recent years. In transfusion medicine, Lewis blood grouping has caused problems because an unaccountable incompatibility between erythrocyte phenotype and saliva Lewis enzyme activity in certain groups of individuals, e.g. pregnant women and cancer patients, has been observed. In cancer, Lewis antigens are often aberrantly or overexpressed and may serve as tumour markers, and Lewis and secretor phenotypes have been associated with the predisposition to infectious diseases and have been implicated as genetic markers/susceptibility factors in multifactorial conditions such as heart disease and peptic ulcers. The sialylated counterparts of the Lewis antigens (sLe<sup>a</sup> and sLe<sup>x</sup>) have been identified as ligands for the adhesion molecules E, P, and L-selectins, and it has been suggested that these tumour-related carbohydrate structures are involved in the haematogenous metastasis of tumor cells.

The focus of this thesis was the investigation of the molecular genetics of the Lewis and secretor genes which encode the Lewis- and secretor fucosyltransferases respectively. The fucosyltransferases control the expression of the two main Lewis antigens Le<sup>a</sup> and Le<sup>b</sup>. The Lewis enzyme is also supposed to direct the synthesis of the tumour marker sLe<sup>a</sup>, also widely known as CA 19-9, and the secretor fucosyltransferase is responsible for the formation of salivary ABH substance.

In bladder cancer, there has been a lack of noninvasive diagnostic and prognostic markers. The present interest in the sLe<sup>a</sup> carbohydrate antigen as a potential marker in bladder cancer is due mainly to the knowledge of the dramatic changes in carbohydrate expression known to occur during the malignant transformation of the urothelium.

### *Aims*

The specific aims were: a) To identify and investigate the mutations that lead to the Lewis-negative phenotype in the Danish population; b) to identify mutations that lead to the nonsecretor phenotype in the Danish population; c) to investigate the mechanism(s) that leads to the non-genuine Lewis-negative phenotype; d) to study the sLe<sup>a</sup> carbohydrate antigen in urine of bladder cancer patients in relation to clinical stage and different Lewis and secretor genotypes; e) to establish novel reference intervals for measurements of circulating sLe<sup>a</sup> in serum based on secretor and Lewis genotypes and to evaluate Lewis and secretor genotyping methods, and f) to study the possible functional role(s) of sLe<sup>a</sup>-rich structures in urine from bladder cancer patients.

### *Results*

The following section briefly summarises the key points of the four studies included in the thesis.

In the first study, the mutational spectrum of the Lewis gene in a Caucasian population was investigated by the use of sequencing, restriction cleavage analysis, cloning and AS-PCR methods. COS7 cells transfected with an allele having the 202/314 mutations which have previously been associated with the Lewis-negative phenotype lacked enzyme activity and cell surface expression of Le<sup>a</sup> structures. Lewis genotypes determined by restriction fragment analysis were compared with the Lewis enzyme activity of saliva and concentrations of circulating sLe<sup>a</sup> in serum of healthy individuals as well as genuine and non-genuine Lewis-negative cancer patients. In healthy individuals, a gene dosage-effect was detected, as *FUT3* heterozygotes had lower  $\alpha$ -1,4-Fuc-T activity and a lower concentration of sLe<sup>a</sup> in serum than homozygous wild-type individuals. The lower enzyme activity in heterozygous individuals seemed to be causally involved, together with the A<sub>1</sub> phenotype, in the conversion of Lewis-positive individuals to non-genuine Lewis-negative individuals during disease periods or during pregnancy.

In the second study, the use of genotype-interpreted measurements of sLe<sup>a</sup>

in the urine of bladder cancer patients was investigated. The concentration of sLe<sup>a</sup> was measured in bladder cancer patients and healthy controls and correlated with T-category, histological grade and presence of urothelial dysplasia. Individuals were Lewis genotyped and secretor status was determined in urine by a WGA-ELISA method. SLe<sup>a</sup> concentrations in urine were higher ( $P < 0.01$ ) in bladder cancer patients than in healthy controls and significantly higher in patients with concomitant urothelial dysplasia than in patients with a normal epithelium. Non-secretor cancer patients had higher concentrations than secretors and again, confirming observations in the first paper, a gene dosage-effect was observed for mutations in *FUT3*.

In the third study, concentrations of sLe<sup>a</sup> in serum and secretor and Lewis genotypes were determined in 500 healthy individuals. The mutational spectrum of the secretor gene in the Danish population was established and a similar gene dosage-effect on sLe<sup>a</sup> concentrations was observed for mutations in this gene. The biological variation of concentrations of sLe<sup>a</sup> was investigated and analytical,  $CV_{A'}$ , within-subject,  $CV_I$  and between-subject  $CV_G$  variability was established as 9.8%, 15.8%, and 102.2% respectively. Genotype-based reference intervals for sLe<sup>a</sup> were established. Based on the comparison with conventional Lewis blood grouping, it was concluded that PCR-based genotyping is an accurate and convenient method to determine secretor status and Lewis histo-blood group.

In the fourth study, urine from bladder cancer patients was subjected to size exclusion chromatography and eluted fractions were analysed for sLe<sup>a</sup> antigen activity. Peak fractions were pooled, concentrated by ultrafiltration and used for cell adhesion experiments. It was found that the binding to E-selectin by a bladder cancer cell line and human neutrophils was inhibited by high molecular weight fractions with high sLe<sup>a</sup> antigen activity. To further study the interaction with E-selectin these fractions were incubated with E-selectin coated into microtitre trays and the bound antigens were detected with an anti-Le<sup>a</sup> antibody.

#### *Conclusions and future aspect*

1. The mutational spectrum of mutations in Lewis and secretor genes in a Danish (Caucasian) population was established.
2. The frequency of *FUT3* heterozygosity is increased in non-genuine Lewis-negative individuals and may be a contributory cause of the non-genuine Lewis-negative blood type.
3. The activity of Lewis enzyme activity in saliva is influenced by Lewis genotype.

4. SLe<sup>a</sup> (CA 19-9) is increased in urine from bladder cancer patients and the highest concentrations are found in patients with concomitant dysplasia of the urothelium.
5. SLe<sup>a</sup> (CA 19-9) concentrations in serum and urine are influenced by secretor and Lewis genotypes.
6. Genotyping of *FUT2* and *FUT3* represents an accurate method for secretor and Lewis typing.
7. Genotype-based reference intervals for sLe<sup>a</sup> are presented and may represent a way to increase the clinical utility of this tumour marker.
8. High molecular weight fractions of urine from bladder cancer patients interact with E-selectin and can inhibit the adhesion of human neutrophils and bladder cancer cells to recombinant E-selectin.

Lewis and secretor blood groups have been associated with a number of different diseases. These studies have all relied on conventional phenotyping of erythrocytes with the immunological detection of single terminal carbohydrate structure. This method cannot compensate for the many different parameters which may influence the complex synthesis of the carbohydrates which carry Lewis antigens as the terminal structure. By virtue of the discovery of the molecular basis for the Lewis negative and nonsecretor phenotype and the following development of straightforward restriction cleavage assays, the biological significance of the Lewis and secretor histo-blood group systems can now be examined properly.

The significant differences in sLe<sup>a</sup> concentrations found in serum of healthy individuals with different secretor and Lewis genotypes indicate that genotype-based reference intervals could improve the use of this tumour marker. Studies of sLe<sup>a</sup> in serum of cancer patients are, however, needed to evaluate whether genotype-based reference intervals will improve the clinical performance of this tumour marker. In general, the increasing knowledge of genetic heterogeneity of protein markers in clinical chemistry may lead to the establishment of genotype-based reference intervals for other markers.

SLe<sup>a</sup> carbohydrate antigen was increased in bladder cancer patients and the highest concentrations were found in those patients who had tumour changes in large areas of the urothelium along with the tumour. Prospective studies with genotype-interpreted sLe<sup>a</sup> measurements on tumour urine are needed to evaluate whether this method is superior to already established methods with respect to the identification of patients with concomitant dysplasia of the

urothelium and a high risk of recurrence. Another line of research is concerned with the further characterisation of the glycoproteins which carry sialylated Le<sup>a</sup> structures in urine from bladder cancer.