

# $\alpha(1,2)$ fucosylation in human colorectal carcinoma

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**Abstract.** Lewis<sup>b</sup> and Lewis<sup>y</sup> (Le) antigens are known to be elevated in colorectal tumours. Alterations in the catalytic behaviour of GDP-L-fucose:β-D-galactoside α(1,2) fucosyltransferase [α(1,2)FT, EC: 2.4.1.69], the key enzyme in their synthesis, have been suggested as being responsible for these changes. In particular, an aberrant tumour-specific α(1,2)FT activity that converts Le<sup>a</sup> and Le<sup>x</sup> to Le<sup>b</sup> and Le<sup>y</sup> determinants, respectively, has been reported in colorectal cancer tissues. To clarify the catalytic function of this enzyme during colorectal tumorigenesis, we analyzed α(1,2)FT activity levels in healthy and tumour colon specimens using different acceptor substrates and determined the kinetic properties of the enzyme. To complete the study, the aberrant Le<sup>a</sup>/Le<sup>x</sup> α(1,2) fucosylation was determined in healthy and tumour colorectal tissues. A correlation analysis between the activity levels and various standard clinicopathological features, such as tumour stage, was also carried out to elucidate the role of these activities in tumour progression. The results obtained confirm the enhanced α(1,2)fucosylation in colorectal neoplastic tissues and the importance of the aberrant Le<sup>a</sup>/Le<sup>x</sup> α(1,2)FT activity in this increase. However, taking into account the high levels of Le<sup>a</sup>/Le<sup>x</sup> fucosylation observed in healthy control tissues, we must rule out the idea of a colorectal tumour-specific α(1,2) FT. On the other hand, no significant association was observed between α(1,2)FT activity levels and the clinicopathological characteristics. Overall, our results suggest that α(1,2)FT activity plays a critical role in the accumulation of Le<sup>b</sup> and Le<sup>y</sup> antigens in human colorectal carcinoma.

## Introduction

Alterations in glycosylation patterns are one of the characteristics associated with differentiation and malignant

transformation (1). The oligosaccharide portions of cellular glycoconjugates, glycolipids and glycoproteins, convey specific immunodeterminants, such as the blood group and related antigens (2). These antigens are known to play an important role in cell recognition, interaction, adhesion and motility. Accordingly, changes in these antigens during carcinogenesis may be crucial in tumour progression and may be involved in extravasation and metastatic phenomena (3).

ABO and related determinants are the result of the sequential addition of monosaccharide units to one of the five disaccharide precursors described. Type 1 [Galβ (1,3)GlcNac β1-R] and 2 [Gal β(1,4)GlcNac β1-R] precursors produce H<sub>1</sub> and H<sub>2</sub> structures, respectively, after the addition of a fucose in α(1,2) linkage to the terminal β-galactosyl residue. H<sub>1</sub> and H<sub>2</sub> determinants can subsequently be converted into Le<sup>b</sup> and Le<sup>y</sup> difucosylated antigens by fucosylation in α(1,4) or α(1,3) linkage, respectively. Their positional isomers, the Le<sup>a</sup> and Le<sup>x</sup> antigens, show a similar structure, but the antigens are not fucosylated in α(1,2) (4). Specific alterations of Lewis antigens have been reported in multiple carcinomas and have been correlated with the progression and malignancy of neoplasms. In particular, in distal colon and rectal carcinomas, the expression of Le<sup>b</sup> and Le<sup>y</sup> determinants has been associated with tumour development and prognosis (2,5). The mechanisms accounting for the role of these carbohydrate antigens in tumour behaviour are unclear, but they have usually been associated with interaction with specific cell receptors. Concordantly, GDP-L-fucose:β-D-galactoside α(1,2)fucosyltransferase [α(1,2)FT, EC: 2.4.1.69], the key enzyme in the biosynthesis of Le<sup>b</sup> and Le<sup>y</sup> antigens, presents increased activity and expression in human colorectal carcinomas (CRC) and cancer cell lines (6-8).

In humans, the functional α(1,2)FTs, described as H and Se, are encoded by two distinct genes, *FUT1* and *FUT2*, respectively (9,10). A third non-functional enzyme is the product of the *FUT2* pseudogene *sec1* (11). The H-type enzyme is mainly expressed on erythrocyte membranes and in vascular endothelium, whereas the Se-type enzyme is found on epithelial cells and in body fluids (12). The catalytic properties of the two enzymes, such as substrate affinity, are also different (13). Finally, the presence in human colorectal carcinomas of an aberrant α(1,2)FT that converts Le<sup>a</sup> to Le<sup>b</sup> and Le<sup>x</sup> to Le<sup>y</sup> is marked; the action of this 'aberrant' activity may explain the accumulation of these structures in CRC (14).

Better knowledge of the molecular mechanisms supporting the expression of tumour-associated difucosylated antigens

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Le<sup>b</sup> and Le<sup>y</sup> may help to elucidate the involvement of these structures in tumour development and progression. Thus, to clarify the biosynthetic pathway that permits the accumulation of Le<sup>b</sup> and Le<sup>y</sup> determinants in colon carcinomas, we determined and characterized the  $\alpha(1,2)$ FT activity in healthy and tumour specimens from patients with CRC using  $\Phi$ - $\beta$ -D-galactoside and Le<sup>a</sup> and Le<sup>x</sup> structures as substrate acceptors.

## Materials and methods

**Tissue specimens.** Colorectal tissue samples were obtained from patients with CRC undergoing surgery at the University Hospital complexes of Ourense and Vigo, Spain, after receiving approval from the appropriate local institutional board. After resection, 23 specimens of primary colorectal carcinoma and histologically healthy tissue (at least 10 cm from the tumour) were washed with ice-cold saline buffer and stored at -85°C until use.

**Preparation of tissue extracts.** Colorectal specimens (0.2–0.4 g) were hand-homogenized in 2–3 volumes of 0.1 M Tris-HCl buffer (pH 7.2), containing 3% (v/v) Triton X-100, in a Potter-Elvehjem device. The homogenate was centrifuged at 5,000  $\times$  g at 4°C for 10 min. The supernatant obtained was centrifuged at 142,000  $\times$  g for 30 min at 4°C. The final pellet, resuspended in 400–800  $\mu$ l of 0.1 M Tris-HCl buffer (pH 7.2), was stored in an ice bath until used as an enzyme source for fucosyltransferase assays. The protein content of the enzyme preparations was determined with the BCA (Bicinchoninic acid; Sigma, St. Louis, MO, USA) protein assay, using bovine serum albumin as the standard.

**GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ fucosyltransferase activity.** To secure the optimal experimental conditions, we first tested the effect on enzyme activity of different donor and acceptor substrate concentrations, incubation times, protein contents and the incorporation of cationic or protecting agents such as ATP, CDP-choline and carbodiimide (Sigma).

Once the assay conditions were optimized,  $\alpha(1,2)$ FT activity was determined using  $\Phi$ - $\beta$ -D-galactoside as an exogenous acceptor and GDP-L-fucose (Sigma)/GDP-L-[<sup>14</sup>C]-fucose (Amersham Bioscience, Europe GMBH) as a donor substrate (10–10.5 GBq/mmol). In the optimized standard assays, the reaction mixture had the following final concentrations in a total volume of 100  $\mu$ l: 50 mM Tris-HCl (pH 7.2), 4 mM NaF, 5 mM MnCl<sub>2</sub>, 3% (v/v) Triton X-100, 10 mM CDP-choline, 100  $\mu$ M GDP-L-fucose/GDP-L-[<sup>14</sup>C]-fucose (isotopic dilution 1/200), 25 mM  $\Phi$ - $\beta$ -D-galactoside and 200  $\mu$ g of protein solution.

Standard assays were run in duplicate for 90 min at 37°C in a shaking water bath. The reaction was terminated with 0.4 ml of cold water and immersion in an ice bath. The reaction product was separated from GDP-L-[<sup>14</sup>C]-fucose by reverse-phase chromatography on Sep-Pack Plus C<sup>18</sup> cartridges (Waters, Milford, MA, USA), using a manifold station coupled to a vacuum pump to reduce the product separation time. After washing with 8 ml of water, the retained fucosylated  $\Phi$ - $\beta$ -D-galactoside was eluted twice with 5 ml of methanol and dried under a stream of nitrogen. Radioactivity was measured on a Wallac 1409-12 Scintillator system, using Ecoscint H as the

scintillation counting mixture. Control incubations without  $\Phi$ - $\beta$ -D-galactoside were performed in parallel to reject the GDP-L-[<sup>14</sup>C]-fucose not specifically bound or transferred to endogenous tissue acceptors. Enzyme activity was expressed as  $\mu$ U/mg protein.

**Determination of  $K_m$  and  $V_{max}$  for GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ fucosyltransferase.** Kinetic studies were carried out for the donor and acceptor substrates under the following conditions: for GDP-L-fucose, donor substrate within the 10–200  $\mu$ M range at a saturating concentration of the acceptor substrate (25 mM  $\Phi$ - $\beta$ -D-galactoside), and for  $\Phi$ - $\beta$ -D-galactoside acceptor within the 1–40 mM range under a saturating concentration of the donor substrate (100  $\mu$ M GDP-L-fucose/GDP-L-[<sup>14</sup>C]-fucose, isotopic dilution 1/200). In both sets of kinetic studies, the previously optimized standard reaction mixture conditions were maintained.  $K_m$  and  $V_{max}$  were determined using the 'Enzfitter' non-linear regression data analysis program (Elsevier, Biosoft).

**GDP-L-fucose:Le<sup>a</sup> or Le<sup>x</sup>  $\alpha(1,2)$ fucosyltransferase activity.** GDP-L-fucose:Le<sup>a</sup> or Le<sup>x</sup>  $\alpha(1,2)$ FT activities were determined as previously described by Yazawa *et al* (14) with some modifications. The standard reaction mixture in a final volume of 50  $\mu$ l had the following final concentrations: 50 mM Tris-HCl buffer (pH 7.2), 5 mM MnCl<sub>2</sub>, 3% (v/v) Triton X-100, 0.5  $\mu$ M GDP-L-[<sup>14</sup>C]-fucose, 99.5  $\mu$ M GDP-L-fucose, 0.5 mM exogenous acceptor (Le<sup>x</sup> or Le<sup>a</sup>) and 100  $\mu$ g of protein solution. Assays were run in duplicate for 90 min at 37°C in a shaking water bath. The reaction was terminated by the addition of 0.4 ml of cold water and immersion in an ice bath. The reaction products were separated from GDP-L-[<sup>14</sup>C]-fucose by anion-exchange chromatography in 3-ml Dowex columns (2 $\times$ 8–400 mesh). Each incubation mixture was placed in a column, eluting with 5 ml of water. The isotopic signal of the fucosylated products was measured as previously described for GDP-L-fucose: $\beta$ -D-galactoside  $\alpha(1,2)$ FT activity. Control assays without acceptors were performed to determine non-specific radiolabelling. The activity results were expressed as  $\mu$ U/mg protein.

**Statistical analyses.** Statistical analyses were performed using SPSS v. 16.0 for Windows XP. The non-parametric tests used were: Wilcoxon, Mann-Whitney U and the Kruskal-Wallis test. The results were considered significant at  $p < 0.05$ .

## Results

**Assay conditions for the determination of GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ fucosyltransferase activity.** In order to optimize the GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$  FT activity assays, different conditions were tested. With respect to the time of incubation, 60, 90 and 240 min were tested. Protein contents of 50, 100 and 200  $\mu$ g were assayed. Furthermore, the effect of different donor and acceptor substrate concentrations in the assay (1–40 mM  $\Phi$ - $\beta$ -D-galactoside and 6.67–100  $\mu$ M GDP-L-fucose) was analysed to select the final saturating conditions. Based on our preliminary results (data not shown), we selected 90 min of incubation and 200  $\mu$ g of protein per assay in order to preserve the maximum

linear response of enzyme activity, as well as 25 mM of  $\Phi$ - $\beta$ -D-galactoside and 100  $\mu$ M of GDP-L-fucose to achieve saturating substrate concentrations.

Another study involved an analysis of the effect of the addition of divalent cations  $Mn^{2+}$  or  $Mg^{2+}$  (0-20 mM) on GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ FT activity. In this sense, no positive effects were found when  $Mg^{2+}$  cations were used in the assay cocktail; however, the presence of  $Mn^{2+}$  cations produced an important increase in enzyme activity. Therefore, we incorporated 5 mM of  $MnCl_2$  as a cofactor in the assay mixture. Furthermore, in order to protect the stability of the GDP-L-fucose complex by means of endogenous tissue pyrophosphatase inhibition, different concentrations of NaF, ATP and/or CDP-choline were tested (data not shown). The greatest protective effect was observed with CDP-choline. Thus, in later determinations, 10 mM CDP-choline was included in the standard assay. Finally, the protective role of carbodiimide against potential fucosidase activities present in the tissue was also tested. In this case, no improvements in enzyme activity were found, and carbodiimide was therefore excluded from the standard incubation.

**GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ fucosyltransferase activity in healthy and colon adenocarcinoma tissues.** Once the optimal incubation conditions had been determined, the evaluation of  $\alpha(1,2)$ FT activity in 18 specimens of healthy and tumour colorectal tissues from the same patient was performed using  $\Phi$ - $\beta$ -D-galactoside (25 mM) as exogenous acceptor. The mean activity was ~5-fold higher in the tumour tissue than the mean value obtained in the control tissue (Table I). The Wilcoxon test indicated that the increase in activity in the tumour specimen with respect to the healthy one was statistically significant ( $p < 0.001$ ).

In order to estimate the usefulness of  $\alpha(1,2)$ FT activity as a diagnostic tool, we developed a correlation analysis between the activity levels and various standard clinicopathological features. Thus, we studied the possible association between the gender and age of the patients with respect to  $\alpha(1,2)$ FT activity. In the case of age, three groups were established on the basis of the 33 and 67 age percentiles ( $<72$ ,  $72-76$  and  $>77$  years). These groups were compared with the activity levels using the Kruskal-Wallis test, and no correlation was found (data not shown). Moreover, the enzyme activity levels were also

Table I. GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ fucosyltransferase activity levels in healthy mucosa and colon adenocarcinoma from patients with CRC.

Tissue	No.	Specific activity	Range	P-value
Healthy	18	$3.13 \pm 0.45$	0.81-7.19	0.001
Tumour	18	$15.59 \pm 2.79$	0.47-33.91	

All assays were performed in duplicate as described in Materials and methods using the conditions optimized in this work. Data are expressed as  $\mu$ U/mg protein and represent the mean  $\pm$  SEM (standard error of the mean). P-value according to the Wilcoxon test.

independent of patient gender according to the Mann-Whitney U test (data not shown). With respect to Dukes' stage, an increase in activity was observed in specimens at advanced stages, B ( $n=5$ ) and C ( $n=8$ ) ( $20.47 \pm 5.52$  and  $16.32 \pm 4.63$   $\mu$ U/mg, respectively), when compared to stage A specimens ( $n=4$ ) ( $10.80 \pm 4.39$   $\mu$ U/mg), although this difference did not reach statistical significance.

**$K_m$  and  $V_{max}$  determination for GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ fucosyltransferase activity in healthy and colon adenocarcinoma tissues.** In order to examine the kinetic properties of  $\alpha(1,2)$ FT in colorectal tumours and their surrounding healthy tissues, we determined the  $K_m$  and  $V_{max}$  values for the donor and acceptor substrates. The dependence of  $\alpha(1,2)$ FT activity on the two substrates followed typical Michaelis-Menten kinetics in both the tumour and control tissues (data not shown). For GDP-L-fucose, saturation occurred at values  $>100$   $\mu$ M, while for  $\Phi$ - $\beta$ -D-galactoside saturation occurred at  $>25$  mM. As shown in Table II, both  $V_{max}$  and  $K_m$  for the GDP-fucose substrate were higher in the tumour than in the control tissues. It appears that  $\alpha(1,2)$ FT in transformed tissue has a decreased affinity for the donor substrate but is more efficient in the catalysis. Additionally, in the case of the  $\Phi$ - $\beta$ -D-galactoside substrate, the  $V_{max}$  showed a moderate increase in tumour specimens, while  $K_m$  remained unaffected. Thus, in this case, the affinity for the acceptor

Table II. Kinetic parameters of GDP-L-fucose: $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ fucosyltransferase activity for the donor (GDP-fucose) and the acceptor ( $\Phi$ - $\beta$ -D-galactoside) substrates.

Substrate	$V_{max}$ ( $\mu$ U/mg protein)		$K_m$	
	Tissue		Tissue	
	Healthy (n=4)	Tumour (n=4)	Healthy (n=4)	Tumour (n=4)
$\Phi$ - $\beta$ -D-galactoside	$6.32 \pm 2.31$	$31.80 \pm 11.20$	$9.06 \pm 4.82$ (mM)	$11.04 \pm 4.30$ (mM)
GDP-L-fucose	$7.20 \pm 1.00$	$118.91 \pm 93.16$	$35.52 \pm 7.04$ ( $\mu$ M)	$169.38 \pm 84.47$ ( $\mu$ M)

$V_{max}$  and  $K_m$  in human colon adenocarcinoma and healthy control mucosa were determined as described in Materials and methods, using GDP-[ $^{14}$ C]-L-fucose/GDP-L-fucose at 10-200  $\mu$ M at fixed 25 mM  $\Phi$ - $\beta$ -D-galactoside, and  $\Phi$ - $\beta$ -D-galactoside 1-40 mM at fixed 100  $\mu$ M GDP-[ $^{14}$ C]-L-fucose/GDP-L-fucose. Values are expressed as the mean  $\pm$  SEM (standard error of the mean).

Table III. GDP-L-fucose:Le<sup>x</sup>  $\alpha(1,2)$ fucosyltransferase and GDP-L-fucose:Le<sup>a</sup>  $\alpha(1,2)$ fucosyltransferase (FT) activity levels in healthy mucosa and colonic adenocarcinoma from the same patient.

Tissue	No.	GDP-L-fucose:Le <sup>x</sup> $\alpha(1,2)$ FT		GDP-L-fucose:Le <sup>a</sup> $\alpha(1,2)$ FT	
		Specific activity	P-value	Specific activity	P-value
Healthy	11	22.95±15.53	NS	22.91±14.47	NS
Tumour	11	36.50±15.61	NS	33.58±12.33	

All assays were performed in duplicate as described in Materials and methods using the conditions optimized in this work. Data are expressed as  $\mu$ U/mg protein and represent the mean  $\pm$  SEM (standard error of the mean). NS, not significant according to the Wilcoxon test.

substrate was equivalent, but the reaction kinetics were faster in tumour tissues. Despite these obvious differences, no statistical significance was found for tumour control comparisons performed by means of the Wilcoxon test (Table II).

*GDP-L-fucose:Le<sup>a</sup> and Le<sup>x</sup>  $\alpha(1,2)$ fucosyltransferase activities in healthy and colon adenocarcinoma tissues.* The alternative synthesis pathway of Le<sup>b</sup> and Le<sup>y</sup> determinants associated with tumour development was evaluated in our study using Le<sup>a</sup> and Le<sup>x</sup> structures as acceptor substrates. The levels of this aberrant activity were measured in 11 specimens of tumour and adjacent healthy tissue (Table III). Notably, these exogenous acceptors afforded higher levels of specific  $\alpha(1,2)$ FT activity than when  $\Phi$ - $\beta$ -D-galactoside was used as an exogenous acceptor substrate. In addition, Le<sup>a</sup>/Le<sup>x</sup>-dependent activity was also higher in tumour than in healthy tissues for the two Lewis acceptors, similar to that which occurred during the 'classical'  $\alpha(1,2)$ FT activity. However, the Wilcoxon test did not detect statistically significant differences when control and tumour alternative activities were compared (Table III).

Moreover, as in the case of the classical  $\alpha(1,2)$ FT activity, the correlation analysis between Le<sup>a</sup> or Le<sup>x</sup>  $\alpha(1,2)$ FT activities and patient characteristics, such as gender or age, or tumour features, such as Dukes' stage, revealed no statistically significant results (data not shown).

## Discussion

Many studies have described the up-regulation of  $\alpha(1,2)$ ,  $\alpha(1,3/4)$  and  $\alpha(1,6)$ fucosyltransferases in different types of tumours and, subsequently, the importance of their fucosylated products in tumour development and progression (15-17). Therefore, the accumulation of Lewis antigens, terminal fucosylated oligosaccharides present in glycolipids and glycoproteins, has been associated with the processes of adhesion and endothelial extravasation of tumour cells, both being important steps that mediate hematogenous metastasis.

In the present study, we investigated  $\alpha(1,2)$ fucosyltransferase activity in terms of kinetic behaviour and activity levels in resected tumour and healthy surrounding tissue specimens from patients with CRC. This enzyme catalyzes the synthesis of H-type structures which, after the action of other glycosyltransferases, produce the blood groups A and B, as well as the Le<sup>b</sup> and Le<sup>y</sup> antigens (6). Le<sup>b</sup> and Le<sup>y</sup> are considered oncofetal determinants for colon cancer

since they are expressed throughout the fetal colon. This expression disappears or decreases strongly in adults but these determinants are re-expressed upon tumour development (18). The alteration of  $\alpha(1,2)$ FT activity has been described in several studies as being responsible for the overexpression of these antigens (14,19). Our findings are in accordance with the above notion in the sense that we observed a striking increase in  $\alpha(1,2)$ FT activity in tumour specimens as compared to the control specimens of healthy tissue. To study this activity, we used three different exogenous acceptors in the enzyme assays. Using  $\Phi$ - $\beta$ -D-galactoside as an acceptor substrate, the 'classical' route of  $\alpha(1,2)$ fucosylation, which allows for the synthesis of Le<sup>b</sup> and Le<sup>y</sup> antigens from H type 1 and 2 structures, was measured. However, an aberrant  $\alpha(1,2)$ FT activity was described in colon tumours allowing for the direct conversion of Le<sup>a</sup> and Le<sup>x</sup> antigens into Le<sup>b</sup> and Le<sup>y</sup>, respectively (14). Therefore, using Le<sup>a</sup> and Le<sup>x</sup> determinants as acceptors, it was possible to determine this 'aberrant' activity.

In the case of the classic  $\alpha(1,2)$ FT activity, we observed a statistically significant increase in the tumour tissue: ~5-fold higher than in healthy tissue. A similar alteration has been previously described in colon cell lines and human tumour tissues (14,20,21). Similarly, strongly increased levels of the mRNA transcript for the  $\alpha(1,2)$ FT enzyme have also been observed in both human colorectal cancer and tumour cell lines (8,22), although the relative contribution of the two enzymes described for this classic activity, H and Se, remains unclear.

It is well-known that these H and Se enzymes have different catalytic properties, similar to those that occur with the different affinities for acceptor substrates. For example, the H enzyme acts equally on the H<sub>1</sub> or H<sub>2</sub> substrates, while the Se enzyme is more efficient using the type 1 structure (23). We observed different kinetic characteristics between the  $\alpha(1,2)$ FT activity detected in tumour tissue and its equivalent in healthy tissue. Thus, the  $V_{\max}$  for the  $\Phi$ - $\beta$ -D-galactoside substrate showed a moderate increase in tumour tissue, while  $K_m$  was fairly similar in the tumours and controls. Therefore, it appears that  $\alpha(1,2)$ FT increases its catalytic potential in CRC, while its affinity for the acceptor substrate is almost the same. Additionally,  $V_{\max}$  and  $K_m$  parameters for the donor substrate, GDP-L-fucose, showed an increase in CRC for both parameters. Therefore, it appears that tumour  $\alpha(1,2)$ FT loses its affinity for the binding of the sugar donor nucleotide complex, while it attains increased catalytic efficiency with respect to

the control. To the best of our knowledge, no previous studies addressing the kinetic characteristics of tumour  $\alpha(1,2)$ FT, and the considerable variability for different healthy tissues as described by  $K_m$  values have been conducted. Consequently, the  $K_m$  calculated for  $\Phi$ - $\beta$ -D-galactoside in our experimental design is similar to that reported for the Se enzyme in colon tissue (24), although we are unable to verify that the increase in classic  $\alpha(1,2)$ FT activity is due to Se  $\alpha(1,2)$ FT. Some studies have demonstrated that the expression of the Le<sup>b</sup> antigen in distal colorectal cancer is mainly caused by the up-regulation of Se  $\alpha(1,2)$ FT, and that the expression of H antigens in the normal and neoplastic colon is dependent on the Se gene (22).

Upon investigation of the biosynthetic pathway of Le<sup>b</sup> and Le<sup>y</sup> glycolipids in cell extracts from gastric cancer Kato III cells, a novel  $\alpha(1,2)$ FT activity was described as being responsible for converting Le<sup>a</sup> into the Le<sup>b</sup> antigen (19). In a later study, Yazawa and colleagues demonstrated the presence of this 'aberrant'  $\alpha(1,2)$ FT in colon cell lines and human colorectal carcinoma using both Le<sup>a</sup> and Le<sup>x</sup> antigens as substrates (14). Since this 'aberrant' activity was scarcely detected in healthy mucosa, it was suggested to be a specific characteristic of oncogenic transformation. Our results confirm the presence of the aberrant  $\alpha(1,2)$ FT activity in CRC tissue, but also in healthy control tissue. In particular, although the difference was not statistically significant, the activity observed in tumour tissue was considerably higher than that observed in healthy mucosa. Subsequently, we consider the activity levels determined in the control specimens sufficient to surmise that this aberrant activity would not be exclusive to transformed cells. Nevertheless, a marked enhancement in Le<sup>a</sup>/Le<sup>x</sup>  $\alpha(1,2)$ FT activity was present in CCR tissue and is partially responsible for the accumulation of difucosylated Lewis antigens in this tissue.

The genetic origin of this 'aberrant'  $\alpha(1,2)$ FT is still unclear. Initially assumed to be a result of Se gene modulation during cancer transformation, it presently appears to be associated with the *FUT3* gene that encodes the  $\alpha(1,3/4)$ FT Lewis enzyme (25).

Once the increase in  $\alpha(1,2)$ FT activities was demonstrated in CRC, we studied their value in tumour prognosis. To accomplish this, the association between tumour  $\alpha(1,2)$ FT activity and standard clinicopathological features was studied. The results indicated that the activity levels were totally independent of patient gender and age. However,  $\alpha(1,2)$ FT activity in the presence of  $\Phi$ - $\beta$ -D-galactoside showed a slight tendency to increase with malignancy. Thus, specimens with Dukes' stage B and C had a higher  $\alpha(1,2)$ FT activity than stage A specimens, although the differences were not statistically significant. In the case of the Le<sup>a</sup>/Le<sup>x</sup>  $\alpha(1,2)$ FT activity, we failed to observe any trend associated with the grade of tumour malignancy. It is well-established that increased  $\alpha(1,2)$ FT activity and the subsequent overexpression of H-difucosylated structures are associated with tumour progression (8,26,27). Most studies have reported low survival in CRC patients with high levels of  $\alpha(1,2)$ FT activity (5,8) or a high expression of its fucosylated products (27). However, recent studies with endothelial and tumour cell lines have demonstrated the inhibition of angiogenesis and metastatic capacity in these cells through the up-regulation of *FUT1* and H<sub>2</sub>/Le<sup>y</sup> determinant expression (17,28). The importance

of sialylated Le<sup>a</sup> and Le<sup>x</sup> antigens in endothelial extravasation via E-selectin is well known (30). The enhancement in  $\alpha(1,2)$  fucosylation in tumour cells appears to induce a decrease in these sialylated structures, thereby reducing their invasive potential (17). Other investigators have suggested that the accessibility of H structures at the cell surface is very important for the motility function and apoptotic cell process (26,29). Similarly, the decrease in  $\alpha(1,2)$ fucosylation in colorectal tumour cells reduces resistance to anticancer drugs and increases susceptibility to UV treatments (7). In light of the importance of these aspects in tumour progression, the monitoring of  $\alpha(1,2)$ FT activity and its catalytic products in patients with CRC can be a useful prognostic tool.

In conclusion, the results presented above confirm an alteration in  $\alpha(1,2)$ FT activity in human CRC. Both the classic [ $\Phi$ - $\beta$ -D-galactoside  $\alpha(1,2)$ FT] and aberrant [Le<sup>a</sup>/Le<sup>x</sup>  $\alpha(1,2)$ FT]  $\alpha(1,2)$ FT activities are higher in tumour tissues in comparison with healthy ones. Thus, increase in this activity is likely to be a tumour-specific phenomenon. Nevertheless, the determination of substantial aberrant  $\alpha(1,2)$ FT activity in healthy colon tissue suggests that this activity can be carried out by a constitutive enzyme in the biosynthesis pathway of Lewis antigens and not by an aberrant tumour enzyme. The malignant transformation may affect the regulation mechanism that modulates the two types of  $\alpha(1,2)$ fucosylation. Evidently, further studies will be necessary to clarify the biological meaning of  $\alpha(1,2)$ FT activity and its catalytic products in tumour development and progression.

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