EUROPEAN JOURNAL OF CANCER 45 (2009) 648–655

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Xanthine oxidoreductase – Clinical significance in colorectal cancer and *in vitro* expression of the protein in human colon cancer cells

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ARTICLE INFO

Article history:
Received 29 July 2008
Received in revised form 2 October 2008
Accepted 15 October 2008
Available online 26 December 2008

Keywords:
Xanthine oxidoreductase
XOR
Colorectal neoplasms
Colon cancer
Prognosis
Survival analysis
Virtual microscopy

ABSTRACT

Xanthine oxidoreductase (XOR) is a key enzyme in degradation of DNA and RNA, and has previously been shown to be decreased in aggressive breast and gastric cancer. In this study, XOR expression was assessed in tissue microarray specimens of 478 patients with colorectal cancer and related to clinical parameters. In addition, we performed *in vitro* studies of XOR activity, protein and mRNA in colon cancer cells (Caco-2). Results from the tissue expression analyses show that XOR was decreased in 62% and undetectable in 22% of the tumours as compared to normal tissue. Loss of XOR was associated with poor grade of differentiation ($p = 0.006$) and advanced Dukes stage ($p = 0.03$). In multivariate survival analysis, XOR was a prognostic factor ($p = 0.008$), independent of Dukes stage, histological grade, age and tumour location. The *in vitro* analyses show that XOR is not measurable in undifferentiated Caco-2 cells, but appears and increases with differentiation. We conclude that XOR expression is associated with histological grade of differentiation and extent of disease in colorectal cancer, and it provides significant prognostic information independently of established factors.

1. Introduction

Xanthine oxidoreductase (XOR) catalyses the final reactions of the catabolism of DNA, RNA and high energy phosphates (ATP, GTP and cAMP) in humans and oxidises hypoxanthine to xanthine and on to uric acid.[1] XOR is an iron-containing metalloflavoprotein that is coded for by a single gene located on human chromosome 2p22.[2,3] XOR is mainly expressed in the cytoplasm of hepatocytes, intestinal enterocytes and goblet cells, vascular endothelial cells and breast epithelium.[4,5]

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.10.036

Hypoxia activates XOR both at the transcriptional and at the post-transcriptional levels, and pro-inflammatory cytokines induce XOR transcription in cell culture.[6,7] The wealth of biochemical information on isolated XOR is in contrast to the lack of knowledge on the physiological function of this enzyme, especially in humans.

In a large population-based cohort of breast cancer, we showed that XOR is downregulated in more than half of the breast tumours studied, and that absence of XOR is an independent predictor of unfavourable outcome.[8] We have also demonstrated that the down-regulation of XOR is common in patients with gastric cancer and associated with unfavourable disease-specific survival.[9]

Mouse colon carcinomas show considerably decreased XOR activities compared with analogous normal tissue,[10] but to date, no previous reports describing the distribution of XOR in malignant colon tumours in humans had been published.

Given that XOR is downregulated in human breast and gastric cancer, and as XOR is strongly expressed in the epithelial cells of the intestine, we hypothesised that the protein may also be differentially expressed in human colon cancer. We examined the expression of XOR in a comprehensive series of patients with colorectal cancer and analysed whether XOR expression is associated with clinicopathological parameters and clinical outcome.

To further study the relationship between XOR and cell differentiation, we evaluate XOR expression *in vitro* in the enterocyte-like Caco-2 cell line, a human colon adenocarcinoma cell line that spontaneously differentiates during cell culture.[11]

2. Materials and methods

2.1. Patients

The study included 643 consecutive patients who underwent surgery for histologically verified colorectal cancer at the Helsinki University Central Hospital in 1989–1998. Tissue specimens suitable for the immunohistochemical evaluation of XOR expression were available in 478 of the patients. Survival data were available for all patients and obtained from patient records, the Finnish Cancer Registry and Statistics Finland. Median follow-up for patients alive at the end of follow-up and with XOR expression data available was 9.0 years (range 0.1–15.4).

The clinicopathological characteristics of the patients in this series are as shown in Table 1. Histological grading was performed according to the WHO criteria.[12]

2.2. Preparation of tumour tissue microarrays

Representative tumour regions in routinely fixed paraffin-embedded samples were defined from H&E-stained sections and marked. Donor tissue blocks were sampled with 1.0 mm punchers using a tissue microarray instrument (Manual Tissue Arrayer 1, Beecher Instruments Inc., Silver Spring, MD, USA). Three cores were cut from each donor block for the tissue microarray blocks. From the tumour samples available, six tissue array blocks were prepared, each containing 80–180 tumour samples. Sections of 4 μm were cut and processed for immunohistochemistry.

2.3. Immunohistochemistry

The antigen was enhanced in Target Retrieval Solution, pH 6.0 (DAKO, Carpentaria, CA) at 95–97 °C for 30 min on routinely processed paraffin sections. The sections were then treated with 3% hydrogen peroxide and XOR protein was detected using a well-characterised rabbit polyclonal anti-XOR antibody.[13] The antibody was diluted 1:50 in Blocking Solution (Powervision, Immunovision Inc., Daly City, CA), and incubated with the samples (overnight at +4 °C). An antimouse-peroxidase polymer (30 min at room temperature) and diaminobenzidine as a chromogen (Powervision) were used for visualisation. Specificity of the XOR localisation was confirmed by staining slides with preimmune serum and without the primary antibodies. Human liver samples were included as positive controls in each staining batch.

2.4. Cell line and culture

Caco-2 cells (from ATCC, Manassas, Virginia) were grown in Dulbecco's modified Eagle's medium with Glutamax (Gibco, Paisley, UK) supplemented with 10% (v/v) foetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin in 5% CO_2 at 37 °C. For *in vitro* differentiation, cells were seeded at 5×10^5 cells/10-cm cell culture dish, medium was changed 1 d after plating and every 2–3 d thereafter.

2.5. Maltase activity

Maltase activity was measured according to the method by Messer and Dahlqvist.[14] To prepare cell homogenates for the assay, Caco-2 cells were washed three times with PBS, mechanically harvested in saline and stored at –80 °C. The cell suspensions were homogenised for 45 s, followed by centrifugation at 15,800*g* for 40–50 min at 4 °C, and the supernatant was stored at –80 °C.

2.6. XOR activity

Total XOR and XO activities were determined as described.[15] Cells were washed three times with PBS, harvested in 50 mM potassium phosphate buffer, pH 7.8, containing 0.5 mM dithiotreitol, 1 mM EDTA, 0.5 μg/ml leupeptin and 0.2 mM phenylmethylsulphonyl fluoride, frozen and thawed twice, and centrifuged at 15,800*g* for 8 min at 4 °C in an Eppendorf centrifuge. Protein concentration was measured with a Bio-Rad DC protein assay (Bio-Rad, Hercules, CA), and the supernatant stored at –80 °C.

2.7. Immunoprecipitation and Western blotting

Proteins (0.8 mg) from Caco-2 cell extracts, harvested as above, were diluted 1:2 with PBS containing 1% nonidet P-40 (v/v), 1 mM EDTA and 50 μl/ml protease inhibitor cocktail (Sigma, St. Louis, MO) (IP-buffer) and incubated with 3 μl of polyclonal human anti-XOR antibodies[13] for 90 min at 4 °C. Subsequently, 50 μl protein G sepharose (Amersham Biosciences, Uppsala, Sweden) was added, and incubation continued for further 90 min. The samples were washed four times with IP-buffer, bound proteins eluted with reducing

Table 1 – Distribution of XOR immunoreactivity according to clinicopathological characteristics in 478 patients with colorectal cancer.

Clinicopathological variable	N	XOR			χ^2	p
		Strong n (%)	Moderate n (%)	Negative n (%)		
All patients	478	80 (16)	295 (62)	103 (22)		
Age at diagnosis						
⩽49 years	57	9 (16)	33 (58)	15 (26)	5.20	0.52
50–64 years	142	27 (19)	88 (62)	27 (19)		
65–74 years	160	20 (20)	102 (64)	38 (19)		
⩾75 years	118	24 (17)	72 (62)	22 (21)		
Gender						
Female	221	38 (17)	126 (57)	57 (26)	4.95	0.08
Male	257	42 (16)	169 (66)	46 (18)		
Tumour location						
Colon	267	38 (14)	166 (62)	63 (24)	3.46	0.18
Rectum	211	42 (20)	129 (61)	40 (19)		
Dukes stage						
A	72	16 (23)	45 (62)	11 (15)	14.3	0.03
B	177	34 (19)	116 (66)	27 (15)		
C	127	18 (14)	73 (57)	36 (29)		
D	102	12 (12)	61 (60)	29 (28)		
Histological type						
Adenocarcinoma	439	73 (16)	274 (63)	92 (21)	0.83	0.66
Mucinous	38	7 (18)	21 (55)	10 (26)		
Not available	1					
Histological grade						
Grade 1	14	6 (43)	8 (57)	0 (0)	18.13	0.006
Grade 2	320	56 (18)	203 (63)	61 (19)		
Grade 3	129	18 (14)	75 (58)	36 (28)		
Grade 4	15	0 (0)	9 (60)	6 (40)		

Laemmli-sample buffer, separated on 7.5% sodium dodecyl sulphate (SDS)–polyacrylamide gels (PAGE), transferred to nitrocellulose membranes and the XOR protein detected as described.[15]

2.8. Ribonuclease protection assay (RPA)

To determine XOR mRNA levels, total RNA was extracted by using the RNeasy Mini kit (Qiagen, Hilden, Germany). Specific RNA was quantified using RPA according to the manufacturer's protocol (RPAIII, Ambion, Austin, TX). ^{32}P-labelled antisense RNA probe was transcribed from a DNA template corresponding to the nucleotides 405–789 of the human XOR cDNA,[16] and 100,000 cpm of the radiolabelled probe was hybridised with 30 μg of RNA overnight at 42 °C. To control for the RNA content, 30,000 cpm of a RNA probe transcribed from human β-actin cDNA (pTRI-actin-human, Ambion) was added to the same hybridisation reaction with the XOR probe. After RNase digestion, the protected fragments were separated on 5% polyacrylamide, 8 M urea gel and exposed to autoradiography films (Curix Ortho HT-L Plus, Agfa, Mortsel, Belgium). As another control for the RNA content, 5 μg of RNA from the corresponding samples was subjected to northern blotting analysis with mouse 18S ribosomal gene DNA probe (Ambion) performed as described.[15] The X-ray films from RPA and northern blotting were scanned and analysed as described.[15]

2.9. Digitisation of stained tissue microarray slides

The tissue microarray slides immunostained for XOR were digitised at a 0.26-μm resolution and made available for viewing on our website (http://www.webmicroscope.net/supplements/xor). For image acquisition, we used a Zeiss Axioskop 2 MOT microscope (Zeiss Gmbh, Göttingen, Germany) equipped with a NeoFluar oil-40× objective, Märzhauser motorised specimen stage (Märzhauser, Wetzlar, Germany) and a CCD camera (Zeiss Axiocam HR). Image acquisition was controlled by the KS400 software (Zeiss). The acquired image files were digitally sharpened and stitched into a single montage file, which was compressed into a wavelet-type image file (ECW format) using the ERMapper software (Earth Resource Mapping Pty). The compressed virtual slides were uploaded to the web server running the Image Web Server software (IWS, Earth Resource Mapping Pty, West Perth, Australia). The virtual slides on the website can be viewed at any magnification level within a standard web browser.

2.10. Scoring of XOR immunostaining

Expression of XOR was evaluated by two of the investigators (N.L. and J.L.). Both investigators were blinded to the clinicopathological data at the time of scoring. Scoring was performed on the digitised whole slide tissue microarrays, by the use of a web-based virtual microscopy platform (http://

www.webmicroscope.net). Cytoplasmic XOR staining intensity was scored as follows: strong = staining comparable to that of normal colorectal epithelial cells; moderate = clearly decreased staining; negative = more than 90% of cancer cells negative for XOR protein. The cut-off for negativity was based on our previous studies on breast and gastric cancer.[8,9]

2.11. Statistical analysis

The associations between XOR expression and other clinicopathological factors were analysed using the χ^2 test or Fisher's exact test in case of low expected frequencies. Life tables were calculated according to the Kaplan–Meier method. Events were deaths due to colorectal cancer, and deaths due to other causes were censored. Survival curves were compared with the logrank test or the logrank test for trend in case of three or more ordered categories. Multivariate survival analyses were performed with the Cox proportional hazards model, and a p-value of 0.05 was adopted as the limit for the inclusion of a covariate. Only variables significantly associated with survival in the univariate analyses were included in the multivariate model. The assumption of proportional hazards was ascertained with complementary log plots. The results from the *in vitro* studies are expressed as means and standard deviations, and means were compared by using two-tailed t-test with unequal variance. Values of $p < 0.05$ were considered significant. All statistical tests are two-sided, and analyses were performed by the use of Stata 10 (StataCorp LP, College Station, TX).

3. Results

3.1. XOR expression in normal colorectal epithelium and in colorectal cancer

In the normal colorectal epithelia, XOR was strongly expressed in the surface epithelial cells. There was no or minimal expression of XOR in the stromal cells of normal colorectal tissue.

Staining of cytoplasmic XOR was scored into three categories in 478 specimens with colorectal cancer. Sixteen percent ($n = 80$) of the tumours showed strong staining for cytoplasmic XOR similar to the XOR expression in the normal colorectal epithelial cells, whereas 62% ($n = 295$) showed moderate staining, corresponding to a decreased XOR expression and 22% ($n = 103$) had lost the cytoplasmic XOR expression (Fig. 1).

3.2. Association between cytoplasmic XOR expression and clinicopathological parameters

Loss of cytoplasmic XOR expression was significantly associated with poor histological grade and advanced Dukes stage. No statistically significant association was found between XOR and age at diagnosis, gender, tumour location or histological type (Table 1).

3.3. Association of XOR expression with colorectal cancer-specific survival

Loss of cytoplasmic XOR expression as compared to the normal colorectal epithelium was significantly associated with decreased cancer-specific survival amongst the 478 colorectal cancer patients (Fig. 2). The five-year colorectal cancer-specific survival in patients with strong or moderate XOR expression was 62% (CI95% 56–66%), compared to 44% (CI95% 34–53%) in those with no XOR staining of the cytoplasm (HR 1.69, CI95% 1.25–2.27, $p = 0.0005$).

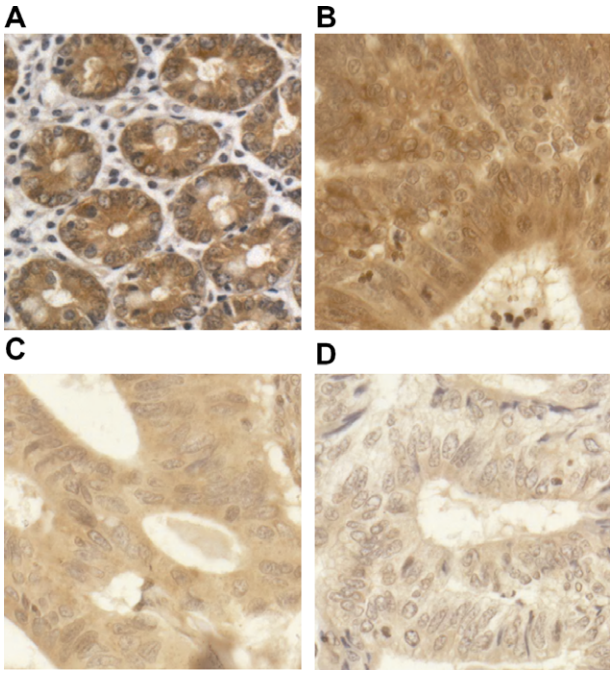

Fig. 1 – Immunohistochemical staining of xanthine oxidoreductase in normal colon epithelium (A) and in colorectal cancer (B–D) as described in Section 2. Examples of strong (B), moderate (C) and no (negative) (D) immunoreactivity in the cytoplasm of tumour cells.

Loss of XOR expression was significantly associated with poor outcome in patients with cancer of both the colon ($p = 0.004$) and rectum ($p = 0.03$), histological grades I–II ($p = 0.03$) and grades III–IV ($p = 0.02$), as well as tumours of adenocarcinoma, not otherwise specified ($p = 0.007$) and mucinous ($p = 0.004$) type (Fig. 2).

3.4. Multivariate survival analysis

Cox multivariate analysis showed that XOR expression (HR = 1.52; 95% CI = 1.12–2.07; $p = 0.008$ for strong and moderate versus. negative) was an independent prognostic factor for colorectal cancer-specific survival after adjustment for Dukes stage, histological grade, age at diagnosis, as well as tumour location (Table 2).

3.5. XOR and maltase activities during Caco-2 cell differentiation

To study the expression of XOR during Caco-2 cell differentiation, XOR and maltase activities were measured from cells cultured for 3, 6, 8, 12 and 15 d. On day 3, XOR activity was not detectable. Confluence was reached on day 5, total XOR (XDH + XO) and XO activities were significantly above the background on day 6 and remained essentially unchanged

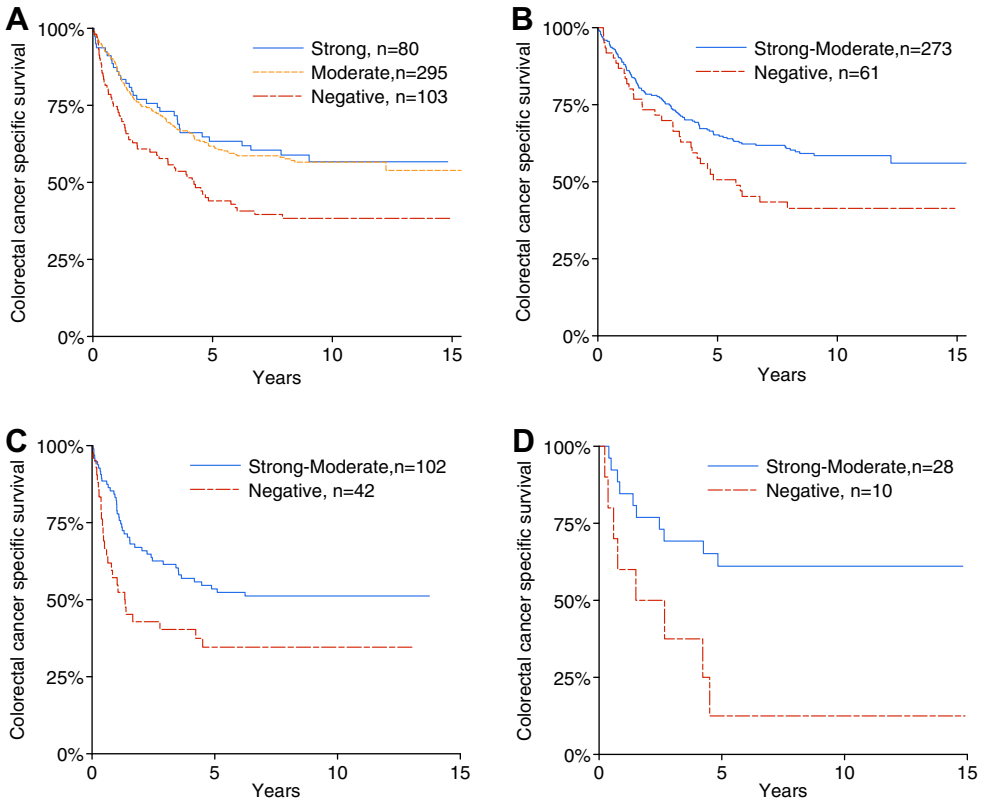

Fig. 2 – (A) Disease-specific survival of 478 patients with colorectal cancer according to xanthine oxidoreductase expression. —, strong ($n = 80$); - - -, moderate ($n = 295$); - — - , negative ($n = 103$). $\chi^2 = 12.2$; $p = 0.0005$ (logrank test for trend). (B) Disease-specific survival of 334 patients with histological grades 1–2 colorectal cancer according to XOR protein expression. —, moderate to strong* ($n = 273$); - - - negative ($n = 61$). $p = 0.03$ (logrank test). (C) Disease-specific survival of 144 patients with histological grades 3–4 colorectal cancer according to XOR protein expression. —, moderate to strong* ($n = 102$); - - - , negative ($n = 42$). $p = 0.02$ (logrank test). (D) Disease-specific survival of 38 patients with colorectal mucinous adenocarcinoma according to XOR protein expression. —, moderate to strong* ($n = 28$); - - - , negative ($n = 10$). $p = 0.004$ (logrank test). *, there was little difference in patient outcome between strong and moderate XOR staining, and therefore these groups were combined and compared with the patients with absent XOR in the survival analysis.

until day 8. Thereafter, XOR activity tripled by day 12 and quadrupled by day 15 (Fig. 3A). XO represented 85% of the increased total activity.

As a marker for small-intestinal enterocyte-like differentiation,[17] maltase activity was determined in parallel cell cultures. An increase in maltase activity was apparent by day 12, and a more than threefold increase was detected between days 3 and 15 of culture (Fig. 3B). These data suggest a relationship between Caco-2 cell differentiation towards small-intestinal enterocyte-like cells and induction of XOR activity.

3.6. *XOR protein and mRNA levels in differentiating Caco-2 cells*

To be able to detect small amounts of XOR protein, Caco-2 cell lysates were immunoprecipitated with anti-XOR antibody. The samples were then subjected to western blotting, followed by enhanced chemiluminescence detection with the same antibody. No band corresponding to the XOR protein was visible in a sample from day 3, whereas a faint band was detectable by day 6, and intensified up to day 15 (Fig. 4A).

XOR mRNA, determined by RPA, was faintly demonstrable on day 3 of culture, but the intensity of the signal increased towards day 15 (Fig. 4B). Levels of β-actin mRNA decreased during cell culture in repeated experiments, rendering β-actin an inappropriate control for RNA content in this setting. When the mRNA for ribosomal 18S gene was determined by northern blotting and used as the basis for quantification, the increase in XOR mRNA was confirmed (Fig. 4B).

The increase in XOR mRNA levels precedes the rise in activity, which is accompanied by the elevation in XOR protein levels, suggesting transcriptional regulation of XOR expression during Caco-2 cell differentiation.

4. Discussion

Here, we show that the downregulation of XOR associates with unfavourable outcome in a series of colorectal cancers. The study comprises the first analysis of XOR expression in colorectal cancer to date, including 478 patients treated at a single centre during a period of 10 years. Moreover, data from *in vitro* studies suggest that XOR is upregulated during differentiation in human colon carcinoma cells.

Table 2 – Cox multivariate regression of the association between XOR immunoreactivity and colorectal cancer-specific survival, adjusted for clinicopathological characteristics (n = 478).

Covariate	HR	CI 95%	*p*-Value
XOR expression			
Strong–moderate	1.00		
Negative	1.52	(1.12–2.07)	0.008
Dukes stage			
A	1.00		
B	1.81	(0.92–3.54)	0.08
C	4.61	(2.41–8.80)	<0.0001
D	30.03	(15.69–57.45)	<0.0001
Histological grade			
1–2	1.00		
3–4	1.50	(1.11–2.02)	0.008
Age at diagnosis			
⩽49 years	1.00		
50–64 years	1.66	(1.02–2.70)	0.04
65–74 years	2.21	(1.39–3.53)	0.001
>75 years	3.31	(2.00–5.47)	<0.0001
Tumour location			
Colon	1.00		
Rectum	1.67	(1.27–2.21)	<0.0001

HR, hazard ratio; CI, confidence interval.

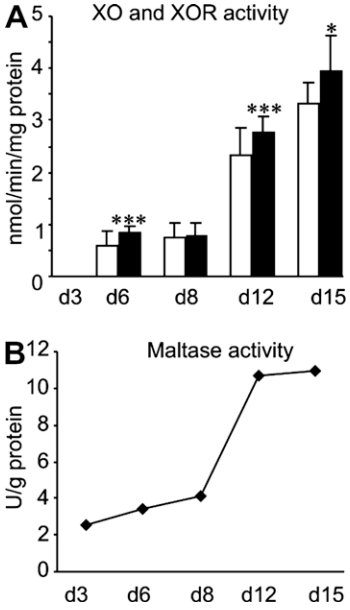

Fig. 3 – Xanthine oxidase (XO), total xanthine oxidoreductase (XOR) and maltase activities during Caco-2 cell differentiation. (A) For differentiation, Caco-2 cells were cultured for 15 d. XO (white bars) and XOR (black bars) activities were measured (see Section 2) in cells cultured for 3 (d3), 6, 8, 12 and 15 d, respectively. Data are means (SD); $n = 5$. $^{***}p < 0.001$, d6 compared with background, d12 compared with d6; $^{*}p < 0.05$ compared with d12. (B) Maltase activity in cells cultured for 3 (d3), 6, 8, 12 and 15 d. Mean values of two parallel determinations of a representative experiment are shown.

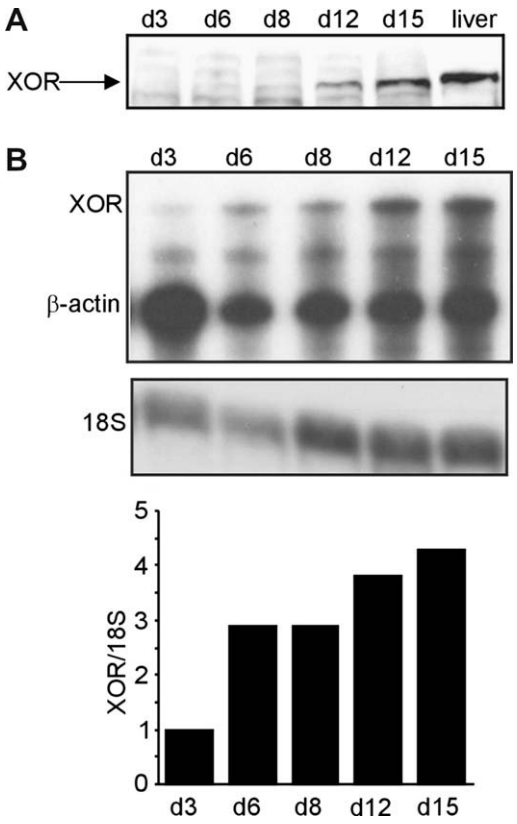

Fig. 4 – Expression of XOR protein and mRNA during Caco-2 cell differentiation. (A) XOR protein was detected in Caco-2 cells cultured up to 15 d (d3–d15). First, 0.8 mg of total protein was immunoprecipitated with anti-XOR antibody, then subjected to western blotting and detection with the same antibody. Arrow depicts the band corresponding to XOR protein. Rat liver extract (liver) was used as a positive control for XOR protein. (B) For ribonuclease protection assay, 30 μg of total RNA, extracted from Caco-2 cells cultured as in A, was used to detect mRNA for XOR and β-actin. To control for RNA content of the samples, 5 μg of RNA was subjected to northern blotting with 18S used as a probe. The amount of XOR mRNA was quantified relative to 18S, and the value for XOR/18S from cells cultured for 3 d was set as one in each experiment. Data are means of two separate experiments.

XOR activity has been detected in a variety of species studied, and in all mammals, including humans; the activities are highest in the liver and in the intestine.[18] Although XOR activity has been determined in a number of animal tumours[19–21] and in a few human tumours,[8,9] little is known about the expression and regulation of XOR in cancer. XOR activity is significantly reduced in rat hepatomas, including the slowest-growing and most well-differentiated tumours.[19] Mouse mammary tumours[22] and colon carcinomas[10] also show considerably decreased XOR activities compared to the corresponding normal tissue. Taken together, the results show that XOR is downregulated in cancerous tissue in virtually all rodent and the human tumours studied so far.

Consistent with the previous studies in breast[8] and in gastric cancer,[9] we observed that a significant proportion of the colorectal cancer samples exhibited either reduction or loss of XOR as compared to the normal epithelia. In breast and in gastric tumours, a reduction of XOR was observed in about half of the samples, whereas more than 80% of colorectal tumours had decreased XOR.

The current study shows that the loss of XOR protein is associated with adverse prognostic characteristics, such as advanced histological grade, advanced Dukes stage, but not with histological type or tumour location. In breast cancer, decreased XOR expression was also associated with several unfavourable features such as poor histological grade of differentiation, large tumour size and high number of positive axillary lymph nodes. Likewise, in gastric cancer, decreased XOR expression was associated with unfavourable features such as high TNM stage, deep penetration depth, lymph node metastases, large tumour size and non-curative disease.

The evaluation of the prognostic significance of the XOR levels in colorectal cancer revealed that patients whose tumour cells had lost the immunoreactive XOR protein had a 70% higher risk of dying from colorectal cancer compared to those whose tumour expressed XOR strongly or moderately. In a multivariate survival analysis, XOR expression was significantly associated with disease-specific survival even after adjustment for conventional variables like Dukes stage, histological grade, tumour location and age at diagnosis. To our knowledge, this is the first report to show an association between XOR expression and patient survival in colorectal cancer.

Our previous study of breast cancer patients showed similar results, *i.e.* a low XOR protein level in the carcinomas was associated with unfavourable survival of the patients. Breast cancer patients with no XOR expression had more than twice the risk of distant recurrence as compared to those with a moderately decreased or normal expression. This was also true in patients with node-negative breast cancer as well as in patients with small tumours.[8] In gastric cancer, decreased XOR expression was significantly associated with poor outcome in patients with early stage disease, negative lymph node status, small tumour size and curative disease.[9]

To date, the molecular mechanisms that regulate XOR expression are poorly understood. Also, the reasons why cancers with decreased XOR expression show aggressive behaviour have not been studied. There appears to be a correlation between upregulation of XOR expression and enterocytic differentiation markers in Caco-2 cells. This cell line provides an opportunity to study the expression of XOR with respect to the degree of cell differentiation. Upon confluence, the Caco-2 cells spontaneously commit towards small-intestinal enterocyte-like differentiation and produce enzymes found in the villus epithelium.[17] We followed the process by measuring maltase activity, including both isomaltase and sucrase activities, which are early markers of small-intestinal enterocyte differentiation.[23] Based on XOR activity, protein and mRNA levels, we observed an induction of XOR expression during culture and enterocytic differentiation of Caco-2 cells. Although the promoter for the human XOR gene has been cloned,[24] little is still known about its transcriptional regulation, and factors determining tissue- and species-specific expression of XOR have not been described. We had previously shown that the ubiquitous transcription factor nuclear factor Y (NF-Y) has an important role in the transcriptional activation of the human XOR gene.[25] A marked induction of the expression of NF-YA, which is one of the subunits of the trimeric NF-Y protein, has been reported parallel to the increasing levels in the markers of small-intestinal enterocyte-like Caco-2 cell differentiation.[26] The transcriptional induction of XOR activity during Caco-2 cell differentiation suggests a link between cellular differentiation and the induction of XOR expression.

As compared to other cancer types, very few molecular prognostic markers have so far been reported in colorectal cancer. Many serum markers for colorectal cancer have been studied, but the only marker of prognostic significance suggested to be added to the established staging systems is CEA.[27] Tumour tissue biomarkers which show prognostic value in colorectal cancer are largely missing, but would be important for clinical decision making on adjuvant therapy. The role of XOR as a potential prognostic marker should be further studied and validated in independent patient series. In summary, this study indicates that the loss of XOR is associated with poor prognosis in colorectal cancer patients, suggesting a tumour suppressor role for XOR in colorectal cancer as had been previously shown for breast and gastric tumours.

Grant support

Cancer Society of Finland, Finska Läkaresällskapet r.f., Medicinska Understödföreningen Liv och Hälsa r.f., Foundation for Pediatric Research.

Conflict of interest statement

None declared.

Acknowledgement

We thank prof. Erkki Savilahti for the maltase activity measurements.

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