Minichromosome maintenance 2 (MCM2) is a new prognostic proliferative marker in Wilms tumour

Katarzyna Taran[1], Anna Sitkiewicz[2], Ewa Andrzejewska[2], Józef Kobos[3]

[1]Department of Pathology, Medical University of Lodz
[2]Department of Oncology and Paediatric Surgery, Konopnicka Memorial Hospital Medical University of Lodz
[3]Department of Pathology of the Age of Development, Konopnicka Memorial Hospital, Medical University of Lodz

Objective: We examined expression of minichromosome maintenance 2 (MCM2) by immunohistochemistry in nephrectomy specimens of children with nephroblastoma treated according to the Society International d'Oncologie Pediatrique (SIOP) scheme to determine its potential prognostic significance.
Material and methods: 18 children with nephroblastoma, 9 females and 9 males, 2 months to 7 years of age, treated in the Department of Oncology and Paediatric Surgery, Medical University of Lodz, during the period 1994-2006 were analysed. Children were treated by neoadjuvant chemotherapy and subsequent nephrectomy according to SIOP protocols -93 and 2001 and followed up from 2 to 11 years. The tumour stage and classification in nephrectomy specimens were established according to the revised 2001 SIOP working classification of renal tumours of childhood.
Results: In low risk nephroblastoma MCM2 expression was low, ranging from 0% in two cases of completely necrotic nephroblastoma to 5% in one child with cystic partially differentiated nephroblastoma. In mesoblastic nephroma, which is a distinct type of neoplasm with a low malignant potential and the most common congenital renal neoplasm, MCM2 expression was variable ranging from 2-5% in 2 children with stage I disease to 70% in one child with stage III disease In intermediate risk nephroblastoma MCM2 expression was low (10%) in one case of regressive type nephroblastoma and stage II disease and intermediate to high in children with epithelial type nephroblastoma, ranging from 40-50% in one case with stage I disease to 70% and 70-100% in 2 children with stage I and stage IV disease, respectively. In high risk nephroblastoma (7 children with nephroblastoma blastemal type) MCM2 expression was intermediate to high, ranging from 40 to 90%. MCM2 expression tends to be lower in children with less advanced stage of disease (stage II) and higher in more advanced disease (stage III and IV). Two children with blastemal type and high (> 60% MCM2) died of disease within 2-4 years from diagnosis and one child was lost to follow-up. Both children who died were older 8.5 yo M and 7 yo M and presented with advanced disease stage IV or III with anaplasia.
Conclusion: MCM2 is a promising prognostic factor in WT treated according to the SIOP scheme.

Key words: Wilms tumour, childhood, MCM2, prognosis.

Introduction

Wilms tumour (WT) or nephroblastoma is the most common malignant renal tumour of childhood, preferentially affecting children below 5 years of age, most commonly during the first 2 years of life. Most WTs present as "triphasic" tumours with three different principal cell types: blastemal, epithelial and stromal cells.

Treatment approaches in WTs are different in North America and Europe but have achieved similar excellent outcome for most cases, with overall survival more than 85%. Large randomized controlled trials have been designed and published by various collaborative groups, including the National Wilms Tumor Study Group (NWTSG), the International Society of Pediatric Oncology (SIOP) and the United Kingdom Children's Cancer Study Group (UKCCSG). According to SIOP guidelines, current standard therapy for most WTs in Europe and other participating countries in the world, consists of preoperative chemotherapy, followed by surgery and adjuvant postoperative chemotherapy or chemotherapy plus radiation depending on histology and tumour stage. The North American NWTSG (National Wilms Tumor Study Group) treatment protocol has an emphasis on immediate nephrectomy followed by adjuvant therapy. In the UKCCSG protocol, a histological diagnosis made on percutaneous cutting needle ("tru-cut") biopsy is required before preoperative chemotherapy [1-3].

The information that can be obtained from pathological examination of treated versus untreated tumours differs in important ways. In the NWTSG trials, both staging and grading criteria can be more precisely evaluated when the effects of therapy have not destroyed or altered tumour structures. However, the SIOP approach provides direct information concerning the responsiveness of a given tumour to the therapeutic agents being used. The SIOP trials have shown that a tumour's response to preoperative chemotherapy is an indicator of good prognosis [4]. Some tumours can show virtually complete ablation of all viable tumour cells after only a few weeks of therapy, while others are more resistant and need more therapy. The presence of a certain amount of blastema after preoperative chemotherapy clearly indicates its nonresponsiveness to chemotherapy and the blastemal type WT has been shown to be associated with poorer outcome. The current 2001 SIOP classification of renal tumours in children stratifies tumours into three risk groups – low, intermediate and high – and defines histological criteria of inclusion for each category. Accordingly, completely necrotic WT has been placed in the low risk tumour group, regressive and epithelial types into intermediate risk and blastemal type into high risk tumour categories. Given excellent results of therapy in WTs the emphasis is now shifting from successful treatment to reducing treatment-associated morbidity without loss of efficacy. The most important prognostic factors in SIOP protocol are tumour histology after pretreatment chemotherapy and tumour stage. In addition, prognosis depends on many other factors related to tumour biology, genetics and immunophenotype, which have been shown to have a prognostic impact in nephroblastoma [5-7].

Evaluation of cell cycle state may predict outcome as have shown studies on many tumours. Proliferation of tumour cells is closely related to their rate of DNA synthesis and may provide prognostic information. An ideal biomarker for evaluation of proliferation should be essential for genomic replication and exhibit a broader range of expression that allows a quick, objective assessment of an individual case. The minichromosome maintenance proteins (MCM), consisting of six members (MCM 2-7), are a family of highly conserved proteins, which form a hexameric complex for regulating eukaryotic DNA duplication and play a central role in S-phase genome stability. MCM expression is upregulated in proliferating cells, providing a diagnostic marker of malignancies [8].

In contrast, remarkably little information regarding its prognostic role is available for solid tumours in children. There have been so far no studies examining the role of MCM2 protein in nephroblastoma.

The aim of the study was to analyse expression of MCM2 in nephrectomy specimens of children with nephroblastoma undergoing therapy according to the SIOP protocol to determine its prognostic significance.

Material and methods

18 patients with nephroblastoma were treated in the Department of Oncology and Paediatric Surgery, Medical University of Lodz, during the period 1994-2006. There were 9 females and 9 males, 2 months to 7 years of age. Children were treated by neoadjuvant chemotherapy and subsequent nephrectomy according to the Society International d'Oncologie Pediatrique (SIOP) protocol -93 or SIOP-2001. Children with clinical stages I-III received prior to surgery actinomycin D and vincristine and with stage IV in addition Adriamycin. Young children age 2 to 19 months were treated by surgery alone. Patients with a high risk of nephroblastoma received post surgery adjuvant chemotherapy or chemotherapy/radiotherapy according to the SIOP protocol. Children were followed up for 2 to 11 years. The tumour stage and classification were established according to the revised SIOP working classification of renal tumours of childhood (2001).

MCM2 expression was analysed by immunohistochemistry (IHC) on paraffin tissue sections of resected tumours using monoclonal anti-MCM2 antibody (Becton Dickinson Transduction Laboratories, Lexington, KY, USA). The staining was performed following antigen retrieval in a water bath at 95 degrees Celsius for 40 min, using Target antigen retrieval solution (DAKO). The sections were incubated with anti-Mcm2 antibody for 1 hour at room temperature followed by mouse Envision+ HRP detection system (DAKO) for 30 min. Peroxidase reaction was developed using liquid DAB (DAKO). Positive reaction resulted in brown nuclear staining in cells expressing MCM2. Positive controls of tonsils showing high MCM2 expression in germinal centres of lymphoid follicles and negative controls using mouse IgG instead of a primary antibody were run in parallel. The percentage of nuclei stained in different tumour components (predominant tumour component) was assessed by a computer image analysis system (Multi Scan Base v. 8.08 – Computer Scanning System, Ltd). All examined microscopic images (Nikon Microphot FXA) were transferred to the computer by camera (CC2OP). Statistical analysis was performed using statistical package SYSTAT for Windows (version 5.03, SYSTAT, Inc. Evanston, Illinois, USA, license No: DA021594). A mean from three 40× high power fields was calculated. For statistical analysis of results the statistical package SYSTAT for Windows (Version 5.03, SYSTAT, Inc) was used. The difference was considered statistically significant at $p < 0.05$.

Results

Histoclinical results

For primary nephrectomy cases, there were 3 cases of mesoblastic nephroma. For pretreated cases according to the SIOP protocol, there were three cases of completely necrotic nephroblastoma, one case of cystic partially differentiated nephroblastoma, three cases of nephroblastoma epithelial type, one case of nephroblastoma regressive type, and 7 cases of nephroblastoma blastemal type.

Three types of the pattern of MCM2 expression were distinguished:
- type I – rare/occasional foci,
- type II – focal,
- type III – diffuse.

Results of low risk WTs according to SIOP 2001

MCM2 expression in low risk nephroblastoma was low, ranging from 0% in cases of completely necrotic nephroblastoma where it was not detectable (3.2-year-old M, stage III, and 11.5-year-old M, stage II) to 5% in cystic partially differentiated nephroblastoma (5-year-old M, stage I). In mesoblastic nephroma, which is a spindle cell renal neoplasm with a low malignant potential and the most common congenital renal neoplasm, MCM2 expression was variable, ranging from 2-3% (8-month F, stage I) to 40% (1-month-old F, stage I) to 70% (2-month F, stage III). The child with a low MCM2 expression (8-month-old F, stage I) suffered from multiple congenital abnormalities and succumbed to heart failure due to left heart hypoplasia but not of disease.

Results of intermediate risk WTs according to SIOP 2001

MCM2 expression in intermediate risk nephroblastoma was variable, ranging from 10% in one case of nephroblastoma regressive type (4-year-old M, stage II) to 40-50% in nephroblastoma epithelial type (4-year-old M, stage I) and was high (70-100%) in another child with nephroblastoma epithelial type (2-year F, stage IV).

Results of high risk WTs according to SIOP 2001

MCM2 expression in high risk nephroblastoma (8 children with nephroblastoma blastemal type) was variable, ranging from 5-7% (7-year-old F, stage II), 15% (2-year-old F, stage II), 40% in 3 children (5.5-year-old F, stage II, 3.6-year-old M, stage III, and 1.4-year-old F, stage II), to high: 60-70% (4.5-year-old F, stage IV), 80% (8.5-year-old M, stage III, nephroblastoma blastemal type with anaplasia) and 90% (7-year-old M, stage IV). Two of the 3 children with blastemal type and high (> 60%) MCM2 died of disease within 2-4 years after diagnosis and one child was lost to follow-up. Both of the children who died were older (8.5-year-old M and 7-year-old M) and presented with advanced disease stage IV or III with anaplasia.

There were statistically significant correlations between histological prognostic markers and MCM2 expression. Details of statistical analysis are presented in Table I.

Discussion

The last few decades of the 20th century saw significant refinements in the management of Wilms' tumour patients, with a considerable reduction in treatment-associated morbidity. The management of most patients with two-agent chemotherapy, with no harmful anthracycline or radiotherapy toxicity, is evidence based and represents curative treatment. However, several areas require further investigation. Relapse Wilms' tumour patients are currently exposed to high-dose treatment regimes, which need

Table I. Correlations between histological prognostic factors in WTs and MCM2 expression

FEATURE I	FEATURE II	PEARSON χ^2	p
Histological typ	type of MCM2 expression	33.919	0.003
	percentage of MCM2 positive cells	28.962	0.016
Histological risk	type of MCM2 expression	16.527	0.057

to be optimized. Further definition of the biological pathways involved in Wilms' tumour and the discovery of novel therapeutic targets should aid the generation of additional therapies.

The assessment of proliferation activity as cell cycle-related protein provides important information on tumour biology and has been shown to have a prognostic impact in WT treated according to SIOP [9].

Minichromosome maintenance 2 is a new proliferation marker and has been shown to be a useful marker in assessing the growth of normal and tumour cells and in evaluating tumour aggressiveness and prognostic value in patients with different types of cancer [10-12]. Our pilot study suggests that MCM2 expression by IHC may be a potential prognostic factor in children with WT treated according to the SIOP protocol due to the presence of statistically significant correlations between MCM2 expression and histological prognostic markers. In low risk WTs MCM2 expression was usually low, in intermediate and high risk groups was variable and higher in children with more advanced disease (stage III and IV). In blastemal type high MCM2 expression was associated with advanced stage of disease and poor outcome and was an adverse prognostic factor for survival and disease progression. High MCM2 in blastemal type (> 60%) was an adverse prognostic factor for survival and may be helpful in predicting a more aggressive clinical course in those children who require more aggressive post-operative therapy to control the disease. In intermediate risk nephroblastoma, MCM2 expression was more heterogeneous, ranging from low to high, and is usually associated with a low stage of disease and younger age. MCM2 expression in intermediate risk WT with an epithelial component seems not to impart adverse prognostic significance, in contrast to blastemal type, which was associated with poor outcome. MCM2 expression may also be helpful in predicting clinical course in low risk nephroblastoma, where MCM2 was usually low and associated with an excellent outcome. Children with low MCM2 might need less adjuvant therapy while children with higher MCM2 expression may benefit from more adjuvant post-surgery chemotherapy or chemo/radiotherapy. Understanding the biology and molecular pathways associated with this aggressive proliferative phenotype of blastemal tumours through gene expression profile may help to design targeted therapy for pathways involved in tumour survival.

The results imply that MCM2 expression may be helpful in assessing WT tumour biology and aggressiveness as determined by response to preoperative chemotherapy. MCM2 was not detectable or low in low risk WT except mesoblastic nephroma, where its expression was variable. However, mesoblastic nephroma is a biologically distinct tumour with a low clinical aggressiveness and should be considered separately from WT. The studies also suggest that high MCM2 expression in the epithelial component may not necessarily be associated with an adverse prognosis. The results of our studies also show that MCM2 expression in nephroblastoma correlates with histological risk groups, according to SIOP.

Our study is in compliance with the results of the SIOP trials and the classification scheme of WT into three risk groups and shows that MCM2 expression is a new prognostic factor in WT [13, 14]. The examinations were carried out on a small group of children with WT treated according to SIOP and further studies are needed to better understand the role of MCM2 expression in tumour biology of nephroblastoma and to assess the clinical usefulness of MCM2 expression in WT treatment protocols [15].

Supported by Grant of Medical University of Lodz No. 502-11-752.

References

1. Vujanic GM, Sandstedt B, Harms D, et al., on behalf of the SIOP Nephroblastoma Scientific Committee. Revised International Society of paediatric Oncology (SIOP) Working Classification of Renal Tumors of Childhood. Med Pediatr Oncol 2002; 38: 79-82.
2. Vujanic GM, Kelsey A, Mitchell C, et al. The role of biopsy in the diagnosis of renal tumors of childhood: results of the UKCCSG Wilms Tumor Study3. Med Pediatr Oncol 2003; 40: 18-22.
3. Vujanic GM, Sandstedt B. The pathology of Wilm's tumor (nephroblastoma): the International Society of Paediatric Oncology approach. J Clin Path 2010; 63: 102-109.
4. Boccon-Gibod L, Rey A, Sandstedt B, et al. Complete necrosis induced by preoperative chemotherapy ins Wilms tumor as

an indicator of low risk: report of the international society of paediatric oncology (SIOP) nephroblastoma trail and study 9. Med Pediatr Oncol 2000; 32: 183-190.
5. Taran K, Kobos J, Sporny S. Examination of expression of WT1 gene product and CD44 adhesive molecule in nephroblastoma histologic types. Pol J Pathol 2008; 59: 177-182.
6. Ghanem MA, Van der Kwast TH, Den Hollander JC, et al. Expression of prognostic value of epidermal growth factor receptor, transforming growth factor-β, and c-erb B-2 in nephroblastoma. Cancer 2001; 92: 3120-3129.
7. Wittmann S, Wunder Ch, Zirn B, et al. New prognostic marker revealed by evaluation of genes correlated with Clinical parameters in Wilms tumors. Genes Chromosomes Cancer 2008; 47: 386-395.
8. Bailis JM, Forsburg SL. MCM proteins: DNA damage, mutagenesis and repair Curr Opin Genet Dev 2004; 14: 17-21.
9. Ghanem MA, Van der Kwast TH, Sudaryo MK, et al. MIB-1 (Ki-67) proliferation index and cyclin-dependent Kinase inhibitor p27 Kip1 protein expression in nephroblastoma. Clin Cancer Res 2004; 10: 591-597.
10. Giaginis C, Georgiadou M, Dimakopoulou K, et al. Clinical significance of MCM-2 and MCM-5 expression in colon cancer: association with clinicopathological parameters and tumor proliferative capacity. Dig Dis Sci 2009; 54: 282-291.
11. Kato H, Miyazaki T, Fukai Y, et al. A new proliferation marker, minichromosome maintenance protein 2, is associated with tumor aggressiveness in esophageal squamous cell carcinoma. J Surg Oncol 2003; 84: 24-30.
12. Obermann E, Went F, Zimpfer A, et al. Expression of minichromosome maintenance protein 2 as a marker for proliferation and prognosis in diffuse large B-cell lymphoma: a tissue microarray and clinico-pathological analysis. BMC Cancer 2005; 5: 162.
13. Kaste SC, Dome JS, Babyn PS, et al. Wilms tumour: prognostic factors, staging, therapy and late effects. Pediatr Radiol 2008; 38: 2-17.
14. Sebire NJ, Vujanic GM. Paediatric renal tumors: recent developments, new entities and pathological features. Histopathology 2009; 54: 516-528.
15. Bukholm IRK, Bukholm G, Holm R, Nesland JM. Association between histology grade, expression of HsMCM2, and cyclin A in human invasive breast carcinomas. J Clin Pathol 2003; 56: 368-373.

Address for correspondence

Katarzyna Taran MD, PhD
Department of Pathology
Medical University of Lodz
Pomorska 251
92-213 Łódz
e-mail: dr.taran.patho@gmail.com