Original paper

Keratin 7 expression in lymph node metastases but not in the primary tumour correlates with distant metastases and poor prognosis in colon carcinoma

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Colorectal carcinoma (CRC) is one of the leading causes of cancer-related deaths worldwide. Alterations in keratin expression, including keratin 7 (K7), are frequent findings in multiple cancers, and they constitute a prognostic factor. The aim of our study was to evaluate the prognostic significance of K7 in the primary tumour and lymph node metastases in two separate cohorts of patients: the first one with lymph node involvement (LN+, 129 cases) and the second one free of LN metastases (LN–, 85 cases).

Keratin 7 expression in CRC was analysed on tissue microarrays with immunohistochemistry and evaluated using the h-score. In the LN+ group K7 positivity was identified in 7/129 (5.4%) of primary tumours (PT) and lymph node metastases (LNM); concordance between them was 94% ($\kappa = 0.396$). Keratin 7 was expressed in 8/85 cases (9.4%) in the LN– group.

K7 expression in LNM of the LN+ cohort correlated with shorter overall survival (OS) ($p = 0.047$) and presence of distant metastases at diagnosis ($p = 0.005$). Expression of K7 in the primary tumour in both cohorts did not correlate with survival. We conclude that the status of K7 expression in metastatic lymph nodes from CRC is a poor prognostic factor.

Key words: keratin 7, metastasis, colon carcinoma, prognosis, lymph node metastases.

Introduction

Colorectal carcinoma (CRC) is the third most common malignancy in humans and the fourth cause of cancer-related deaths [1]. Patients' overall survival is dependent on several factors, e.g. tumour grade and stage, including lymph node involvement [2, 3]. Colorectal carcinomas with lymph node metastases (LNM) differ in many aspects from those that do not present nodal dissemination. The former show worse prognosis, a higher recurrence rate, and shorter overall and disease-free survival [4, 5]. Additionally, application of adjuvant systemic therapy also depends on the lymph node involvement [6].

The tumour cell characteristics of LNMs differ in many aspects from those in the primary tumours (PT) in many human malignancies, e.g., epithelial to mesenchymal transition (EMT) markers in breast carcinoma were more strongly expressed in metastatic lesions [7]. In addition, EMT phenotype more strongly correlated with prognosis if the LNM profile rather than that of the primary tumour was taken into account [8]. In 53% of colorectal carcinomas the histological grade differs between primary tumours and their respective LNMs [4]. However, the other cellular characteristics between the primary tumour and secondary deposits in CRC have not been studied extensively.

Keratins (Ks) form intermediate filaments that are characteristic for epithelial cells. Their family is composed of more than 80 types of proteins, which form hetero-dimers, composed of one molecule of type I K and another one of type II K [9]. Normal epithelial cells usually have a specific keratin profile that differs among various histological types of epithelium. Neoplasms largely retain the keratin profile during malignant transformation, and this feature is widely used in the histological differential diagnosis of carcinomas. Aberrant expression of Ks confers a poor prognosis for the patients, e.g., K8/18 in oral squamous cell carcinoma [10] and oesophageal carcinoma [11], K7 in oesophageal carcinoma [12] and K19 in hepatocellular carcinoma [13]. K20 is constantly expressed in the colon epithelium and thus it is a marker of tissue derivation in CRC. In contrast, K7 is not expressed in the normal colonic epithelium and its expression is an exceptional finding in CRC. However, some clinicpathological subtypes, e.g., BRAF-mutated microsatellite stable or ulcerative-colitis associated CRCs, show much more frequent K7-positivity [14, 15].

In CRC, K7 expression in PT was substantially more frequent in tumours that disseminated to the LN (25.3%) compared to cases without LN involvement (17.3%) [16]. In addition, K7-positivity was associated with shorter survival [17]. However, to the best of our knowledge, no studies comparing K7 expression and its prognostic impact in PT versus their LNM in CRC have been published so far.

The aim of our study was to evaluate the prognostic significance of K7 in two independent cohorts of patients with CRC: one with and another without LN involvement. Additionally, we decided to compare the expression of K7 in PT and LNM in CRC to verify their prognostic value.

Material and methods

All cases were retrieved from the files of the Department of Pathomorphology, Medical University of Gdansk, Poland and the Centre of Oncology, Bydgoszcz, Poland These patients were operated on the Surgical Clinics in the years 1998-2004. The first cohort consisted of 129 patients with CRC disseminated to the regional lymph nodes (LN+). It included 54 females (42%) and 75 males (58%), with the mean age of 63.4 years (range 32-91). The mean follow-up period was 36.6 months (range 19-148.6).

The second cohort of LN-negative CRC (LN–) consisted of 85 patients (50 males and 35 females) with mean age of 66 years (range 32-87) and mean follow-up of 59 months (range 19-143). In 40 patients of this 85 LN– group, the tumour relapsed as a metastatic disease (lungs, liver, bones and skin) during the first three years following surgery. None of the patients received neoadjuvant chemotherapy. We decided to exclude rectal carcinoma from our analysis. Therefore, the cases of resected tumours from the large intestine only (sigmoid, traverse, right-side colon, left-side colon) were taken into account.

Basic clinic-pathological characteristics of both groups are shown in Table I.

Tissue microarrays

Tissue microarrays (TMAs) were constructed from the archival formalin-fixed, paraffin-embedded tissue blocks using a manual tissue arrayer (Beecher Instruments, MTAI, K7 BioSystems).

In the LN+ group, 3 areas rich in tumour cells from each case were punched from the PT and metastatic LN and transferred to the recipient block. In the case of multiple metastatic lymph nodes cores were taken haphazardly from up to three involved lymph nodes In the LN– group two areas of PT were taken from each case.

Immunohistochemistry

Immunohistochemical staining was performed on a DAKO Autostainer with antibody against K7 (DAKO, Clone OV-TL 12/30, ready-to-use).

Intensity of the immunohistochemical reaction was evaluated using the h-score system. In h-score, the respective intensity grades of reaction and the percentage of positive cells were multiplied and summed up (1x n%+2xn%+3xn% = y). Therefore, theoret-

Table I. Basic clinic-pathological characteristics of LN+ and LN– patient cohort

Variable	Cohort 1 LN+								Cohort 2 LN–				
	Number of cases (N)	%	PT			LNM			Number of cases (N)	%	PT		
			K7– (N)	K7+ (N)	p	K7–	K7+	p			K7– (N)	K7+ (N)	p
	129	100	122	7		122	7		85	100	77	8	
Sex													
men	75	58	70	5	0.46	70	5	0.46	50	59	47	3	0.19
women	54	42	52	2		52	2		35	41	30	5	
pT													
pT1	0	0	–	–	0.95	–	–	0.95	40	47	35	5	0.36
pT2	1	1	1	0		1	0		47	53	42	3	
pT3	106	82	100	6		100	6		0	0	–	–	
pT4	22	17	21	1		21	1		0	0	–	–	
pN													
pN0	0	0	–	–	0.62			0.77	85	100	77	8	–
pN1	67	52	64	3		63	4		0	0	–	–	
pN2	62	48	58	4		59	3		0	0	–	–	
pM													
pM0	106	82	101	5	0.44	103	3	**0.005**	85	100	77	8	–
pM1	23	18	21	2		19	4		0	0			
Grade													
G1	8	6	8	0	0.29	8	0	**0.049**	16	19	13	3	**0.046**
G2	94	73	90	4		91	3		55	65	53	2	
G3	27	21	24	3		23	4		14	16	11	3	
Histological type													
tubular	116	90	110	6	0.7	109	7	0.36	74	87	68	6	0.62
mucinous	13	10	12	1		13	0		6	7	5	1	
medullary	–	–	–			–	–		4	5	3	1	
serrated	–	–	–			–	–		1	1	1	0	

ically the score ranged from 0 to 300. The tumour tissue from all cores of a single case was evaluated, separately for PT and LNM, if present. The cytoplasmic reaction was regarded as positive K7 expression. For statistical purposes all cases with any level of K7 expression were referred to as positive. Each case was evaluated independently by two pathologists (P.C. and A.G.).

Statistics

Statistica software (version 12, StatSoft) was used for the analysis. χ^2 or Fisher's exact test was applied for categorical data, where appropriate. The Kaplan-Meier estimator was employed for survival analysis, and the generated curves were compared with Cox's F-test. The endpoint for the study was overall survival (OS). OS was defined as the time from sample collection to death or censoring. Censoring was defined as loss of follow-up or alive at the end of follow-up. Statistical significance was assumed when $p \leq 0.05$. κ, being a measure of the strength of agreement, was calculated using MedCalc software (version 12.5.0.0).

Results

Keratin 7 expression is infrequent in colorectal carcinoma and shows low concordance between PT and LN metastases

In the LN+ group seven PTs (5.4%) and seven LNMs displayed K7 expression in the PT and in the lymph node metastases. In three cases K7 positivity was identified both in PT and LN, and in four cases expres-

Table II. Expression of K7 in PT and LN. Conversion rates from negative to positive: (–) → (+), and positive to negative (+) → (–) status between PT and LNM are given as number of cases and percentages of the total samples number. κ coefficient of concordance is given with 95% confidence interval (CI). N – number of cases

Marker	N	Positive in PT	Positive in LN	Conversion PT → LN			κ coefficient (95% CI)
		N (%)	N (%)	(–) → (+) N (%)	(+) → (–) N (%)	Switch total N (%)	
Cohort 1							
K7	129	7 (5.4)	7 (5.4)	4 (3)	4 (3)	8 (6)	0.396 (0.059-0.73)
Cohort 2							
K7	85	8 (9.4)	–	–	–	–	–

sion was found either in PT or LN. Overall concordance between PT and LN was 94% (κ = 0.396) (Table II).

Representative examples of K7 expression in PT and LNM are shown in Figures 1A,B and 2A,B.

Prognostic significance of keratin 7 expression

Keratin 7 expression in LNM correlated with shorter OS (p = 0.047) (Fig. 3). However, the survival was not altered in cases with K7 expression in PT (p = 0.3) (Fig. 4).

Distant metastases were more frequent in patients showing K7-positive LNM (4/23 vs. 3/104, p = 0.005), whereas the status of K7 in PT was not associated with metastatic dissemination (p = 0.44).

Keratin 7 expression was not different between analysed cohorts (LN+, LN–). K7 was expressed in eight cases (9.4%) in the LN– group, and this feature was associated neither with recurrence of disease (p = 0.26) nor with the overall survival of the patients (p = 0.23). (supplementary material).

Keratin 7 and clinic-pathological factors

Among the features analysed K7 expression was more frequent in grade 3 tumours in the LN– (p = 0.046) cohort. In the LN+ cohort K7 expression in LNM was associated with grade 3 (p = 0.049), while there was not such a correlation for PT (p = 0.29). Keratin 7 expression was not associated with sex, tumour stage, LN status in the LN+ cohort or histological type.

Discussion

In our study K7 expression in CRC was uncommon regardless of the LN involvement by cancer (LN+ vs. LN–, 5.4% and 9.41%, respectively). This is generally in line with the findings of the large study by Harbaum *et al*., who found K7 expression in 9% of unselected PT in a group of 370 patients [17]. However, the percentage of K7+ tumours can be much higher in certain molecular subtypes of CRC, e.g., K7 is expressed in 39% of cases in BRAF-mutated microsatellite-stable CRCs [14]. Similarly, pathogenic background may also determine this feature, since K7 positivity was identified in up to 59% of ulcerative colitis-associated CRC [15]. Seemingly, our cohorts of unselected CRC did not have overrepresentation of these clinic-molecular subgroups.

Interestingly, in a study of Bayrak *et al*., K7 expression in PT was more frequent in LN+ (25.3%) than

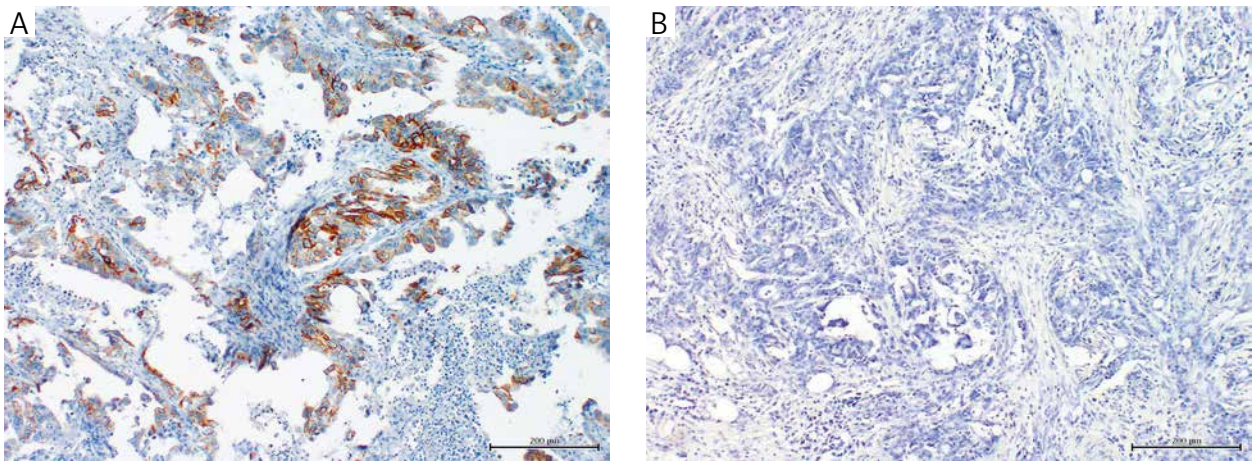

Fig. 1. Positive K7 expression in primary tumor (A) and negative in lymph node metastases (B) in LN+ group

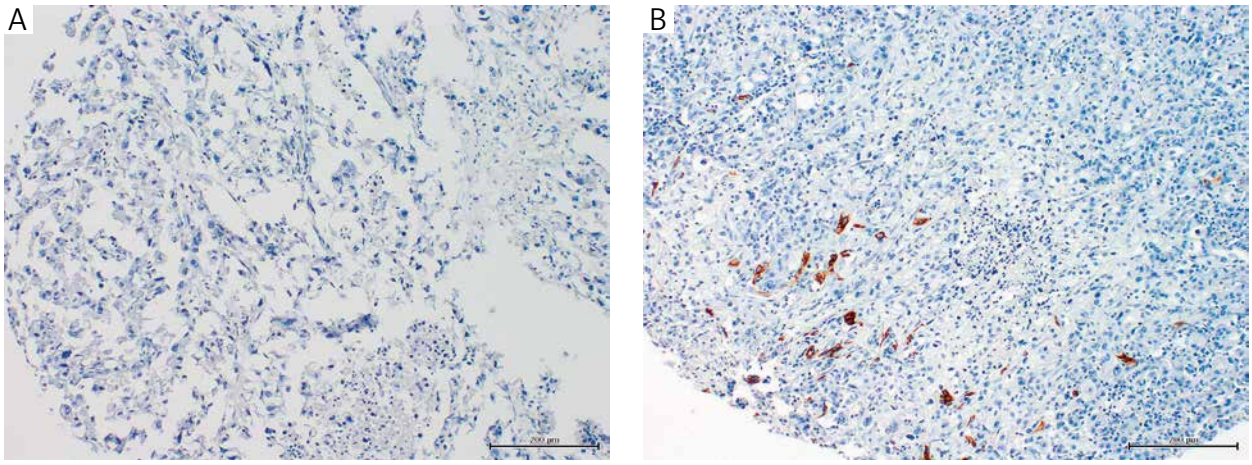

Fig. 2. Negative K7 expression in primary tumor (A) and positive in lymph node metastases (B) in LN+ group

in LN– (11%) CRC patients [16]. We did not observe such differences in K7 expression between these cohorts. So far, data about expression of K7 in LNM of CRC have not been reported. We are the first to show that K7 positivity in LNM occurs infrequently, with similar incidence to that present in PT (5.4%). Aberrant expression of keratins is a poor prognostic factor in multiple tumour types [10, 13], e.g., K7 correlates with an unfavourable clinical course in oesophageal carcinoma [12]. However, data about the poor prognostic role of K7 are limited. The only study that shows worse survival in CRC is the analysis of a large cohort performed by Harbaum *et al.* [17]. They found that 48% of patients with K7-positive tumours died of the disease compared to 33% with K7-negative tumours ($p = 0.06$). Despite the large size of the cohort (370 cases), the diffrenece was not statistically significant. This supports to some extent our observations indicating that expression of K7 in PT tumour is not a prognostic factor in CRC. However, we found that a potential poor prognostic factor is expression of CK7 in local nodal metastases of CRC.

In general, there is a high level of discordance in the expression of markers between PT and LNM in CRC. This concerns p53 and c-myc [18], microsatellite instability (MSI), CpG island methylator phenotype (CIMP) [19], p21, cyclin D1 [20] and thymidylate synthase [21]. IHC expression of tyrosine phosphatase type IV A member 3 (PTP4A3) was found in 18.4% of primary tumours and 91.6% of LN metastases. Mutations in p53 were also more frequent in LNM than in PT [22]. In LNM of CRC expression of markers responsible for more aggressive clinical behaviour is frequently stronger than in PT. This is the case with p53 [18, 23] and the Ki-67 index [23].

Expression of many markers in LNM, including thymidylate synthase [24], epidermal growth factor receptor (EGFR) [25] and p16 [26], shows better

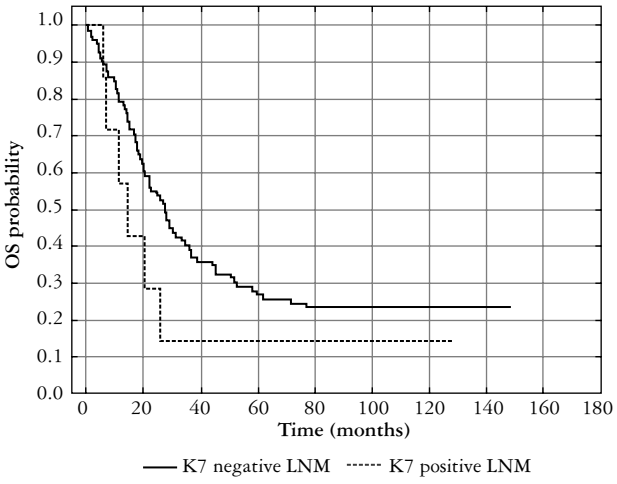

Fig. 3. Kaplan-Meier overall survival curve of LN+ cohort depending on the K7 status in LN

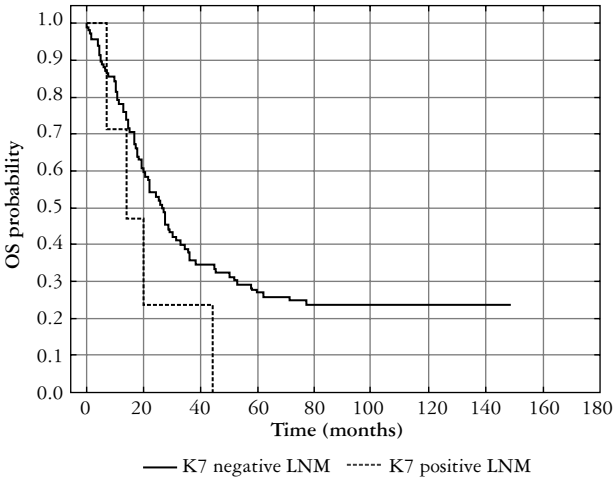

Fig. 4. Kaplan-Meier overall survival curve of LN+ cohort depending on the K7 status in PT

prognostic information in LNM than in PT of CRC. Similar observations were found in other cancers, for example in breast carcinoma [8].

In our LN+ group we observed a strong correlation between K7 positivity in LNM and the presence of distant metastases at the time of diagnosis ($p = 0.006$). This observation also confirms higher biological aggressiveness of tumours expressing K7 in LNM. Indeed, poor prognosis in these patients may be secondary to higher clinical stage of these tumours; however, multivariate analysis did not give support to such a conclusion. On the other hand, an analysis of the population of tumour cells that had disseminated to secondary sites provides additional information on the more aggressive component of the neoplasm. Identification of a potential prognostic marker in this selected clone may provide more precise data about the clinical course of the disease.

As K7 expression in LNM correlates with presence of distant metastases it would be interesting to investigate the expression of K7 in distant metastases. Unfortunately, this material is not readily available. Further studies comparing expression of potential markers, including K7 in PT, LNM and distant metastases of CRC could shed more light on the mutual relationships of these proteins in different tumour locations.

Conclusions

To summarise, we have described K7 expression in LNM but not in PT as a poor prognostic factor in CRC. K7 expression in LNM but not in PT correlates with presence of distant metastases in CRC. Low concordance in the expression of K7 between PT and LNM and its different prognostic influence confirms data on substantial changes in the biology of cancer cells in PT and LNM of CRC.

This study was supported by Grant No. ST-95 from the Medical University of Gdańsk.

PC and MB were supported by the programme "Mistrz" from the Foundation for Polish Science.

P.C., M.B. and M.S. were supported by Grant No. 2011/03/b/nz5/00519 (OPUS 2) from the National Centre of Science.

The authors declare no conflict of interest.

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