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Is ERCC1 a prognostic biomarker for urothelial cancer following radical cystectomy? A long-term analysis

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Introduction Excision repair cross-complementation 1 protein (ERCC1) plays a vital role in cancer cells enabling DNA repair via nucleotide excision repair. Thus, we hypothesized whether expression of this protein may be utilized as a prognostic marker in patients after radical cystectomy.

Material and methods The final analysis involved 123 patients with urothelial bladder carcinoma who underwent radical cystectomy with bilateral lymphadenectomy. The median follow-up time was equal to 853 days. ERCC1 status was evaluated immunohistochemically with the application of tissue microarrays.

Results Positive ERCC1 expression was noted in 46% of the studied cases. Among the analyzed clinical and pathological factors, we could not establish a statistically significant correlation with ERCC1. Similarly, survival curves were statistically indifferent in patients with tumors categorized according to both expression categories. We did not confirm a prognostic value of ERCC1 in the multivariate regression analysis.

Conclusions ERCC1 expression does not influence the overall survival of patients with urothelial bladder carcinoma after radical cystectomy.

Key Words: radical cystectomy ‹› bladder cancer ‹› urothelial carcinoma ‹› ERCC1 expression

INTRODUCTION

Bladder cancer (BC) is the second most common malignancy of genitourinary origin [1], with an estimated 430 thousand new cases diagnosed annually [2]. According to the estimates of the world health organization, the incidence and the mortality of BC is expected to double in the following decades [3].

The most prominent histopathological type is urothelial carcinoma, which is confined to the superficial mucosal layer of the bladder in 70% of cases, i.e. non-muscle invasive bladder cancer (NMIBC). NMIBC can be managed conservatively via endoscopic route and is usually associated with a good prognosis, although the rate of recurrence and progression is significant [4]. The remaining type of BC invades muscular or deeper vesical layers, which is termed muscle invasive bladder cancer (MIBC). Radical cystectomy (RC) with bilateral pelvic lymphadenectomy remains the gold standard for the management of MIBC [5]. Despite recent therapeutic advances, RC is associated with substantial perioperative mortality and a high rate of serious side effects, reaching up to 28% [6]. The 5-year survival rate is also beyond satisfactory and does not exceed 60% [7]. Currently, prognostic factors after RC are based on classical

clinical and pathological characteristics, which are only available after surgical extirpation. Literature data suggest that MIBC is a heterogeneous disease with varied clinical outcomes [8], thus radical treatment may be ineffective in some patients. This necessitates the search for novel biomarkers in order to ensure that aggressive tumors are not misinterpreted, without over-treating less aggressive cancers at the same time.

Excision repair cross-complementation 1 protein (ERCC1) plays a crucial role in maintaining genomic stability. It forms a complex with XPF1 endonuclease, which is a core component of nucleotide excision repair (NER) machinery [9]. NER serves as a primary mechanism for DNA repair in cancer cells exposed to platinum-based chemotherapy [10] and ERCC1 overexpression has been noted in cisplatin-treated, as well as cisplatin-resistant cells [11, 12]. Since pioneering studies confirming the prognostic role of ERCC1 in lung cancer, the prognostic and predictive value of this protein has been extensively studied in numerous cancer types. In terms of BC, ERCC1 has been evaluated mainly in the context of neoadjuvant chemotherapy. Nevertheless, literature data concerning the prognostic value of ERCC1 in BC are limited. Thus, the main purpose of this analysis was to evaluate whether expression of ERCC1, assessed by immuhohistochemistry (IHC), correlates with overall survival (OS) of BC patients following radical cystectomy.

MATERIAL AND METHODS

Study cohort and clinical samples

All patients who underwent radical cystectomy with bilateral lymphadenectomy in a single institution – Holycross Cancer Centre in the years 2012–2013 were retrospectively recruited to the study. A total of 19 patients with T0 tumors or tumors histopathology different than urothelial BC were excluded from the final analysis.

The median time from histologically-confirmed diagnosis to RC was 84 days. The analysis also involved the presence of a main etiologic factor – smoking, which was declared by 41% of the study participants. The presence of at least one significant comorbidity, including diabetes, coronary artery disease, arterial hypertension, or renal failure was noted in 60.2% of patients. The performance status was assessed preoperatively according to the ECOG (Eastern Cooperative Oncology Group Scale of Performance Status) and 64% of patients were categorized as fully active (ECOG – 0). Patient characteristics are summarized in Table 1.

Table 1. *Patient characteristics according to ERCC1 expression category*

Characteristic	ERCC1 negative N = 65[1]	ERCC1 positive N = 58[1]	p-value[2]
Patient age			0.4
Below 65	26 (40%)	28 (48%)	
65 and above	39 (60%)	30 (52%)	
Sex			0.8
Male	54 (83%)	49 (84%)	
Female	11 (17%)	9 (16%)	
T-stage			0.4
T1-T2	15 (23%)	17 (29%)	
T3-T4	50 (77%)	41 (71%)	
N-stage			0.7
N0	33 (51%)	31 (54%)	
N+	32 (49%)	26 (46%)	
Unknown	0	1	
Grade			0.8
Low-grade	6 (9.7%)	4 (7.7%)	
High-grade	56 (90%)	48 (92%)	
Unknown	3	6	
Angioinvasion			0.6
Absent	21 (38%)	21 (43%)	
Present	35 (62%)	28 (57%)	
Unknown	9	9	
ECOG performance			0.8
0	41 (63%)	38 (66%)	
1 and above	24 (37%)	20 (34%)	
Comorbidities			0.5
0	24 (37%)	25 (43%)	
1 and above	41 (63%)	33 (57%)	
Chemotherapy			0.2
Not-performed	37 (57%)	26 (45%)	
Performed	28 (43%)	32 (55%)	
Surgical technique			0.015
Laparoscopic	54 (83%)	56 (97%)	
Open	11 (17%)	2 (3.4%)	

[1] n (%)
[2] Pearson's Chi-squared test; Fisher's exact test
ECOG – Eastern Cooperative Oncology Group

Tissue microarray construction and immunohistochemistry

Surgical specimens underwent routine pathological processing and examination and were archived at the Department of Pathology. Tissue microarrays (TMA) were constructed from archived pathological specimens according to the method described by Konen at el. [13]. ERCC1 expression was assessed by immunohistochemistry with the application of the EN VISION HRP system (Dako agilent). The primary antibody used in the study was the mouse anti-human ERCC1 antibody (Clone 4F9, Dako agilent). Tonsillar tissue served as a positive control and was included in every TMA slide,

whereas negative controls were incubated without the primary antibody. IHC staining was assessed in a semi-quantitative manner, by an experienced pathologist, according to the German Immunohisocemical Scoring System [14]. To ensure reliability, random samples were repetitively analyzed by another pathologist. Intensity of staining was classified as 0 – negative, 1 – weak, 2 –moderate, 3 – strong (equivalent to the positive control). In case of heterogeneous staining, the dominant pattern was noted. ERCC1 expression was expressed as the percentage of positive cells in four high power fields (400x), which is equivalent to approximately a 1 mm^2 surface. Median expression of ERCC1 in the entire cohort was 90%, which was utilized as a cut-off value to discriminate low and high expression categories.

Statistical analysis

The survival time was calculated from the date of the surgery until the date of death or last follow-up, which was set for the 31st of November 2018. For continuous variables median values and interquartile ranges were presented. Absolute values and percentages were shown for categorical variables. The probability of 5-year survival was assessed using the Kaplan-Meier estimator and survival curves were subsequently compared with a log-rank test. To estimate the risk of all-cause death the Cox proportional hazard model was applied and the results are expressed as hazard ratios (HR) with corresponding 95% confidence intervals (95% CI). To examine the association between ERCC1 expression and clinical-pathological features, the Fisher's exact test was utilized.

P values <0.05 were considered significant. All analyses were performed in R software (version 3.4.4) including 'survival' and 'survminer' packages.

RESULTS

The final analysis involved 123 patients with a median follow-up of 853 days. The probability of 5-year overall survival was 27.7%, whereas median survival time was equal to 22.9 months.

Bladder-confined disease (T1 and T2) was noted in 26% of patients, whereas the remainder had extravesical invasion (T3 and T4). Node metastases (N+) were present in 47.2% of study participants, whereas lymphovascular invasion (LVI) was noted in 51% of cases. 84% of tumours were represented by high-grade urothelial carcinomas, whereas 8% of the tumours were low-grade. Histological grade could not be established in the remaining specimens.

Median expression of ERCC1 in the entire cohort was equal to 90%, which was utilized as a threshold value to discriminate between positive and negative expression categories. Representative microscopic images of both positive and negative ERCC1 expression are presented in Figure 1. Positive expression of the studied biomarker was noted in 57 cases, which represents 46% of the studied cases.

Among analyzed clinical and pathological characteristics, statistically significant correlation with ERCC1 expression could not be found. Patients' characteristics divided according to ERCC1 expression categories are summarized in Table 1.

In order to analyze the probability of overall survival, we employed the Kaplan-Maier method. The probability of 5-year OS in patients with ERCC1 positive tumours was 0.329 (95% CI: 0.220–0.491),

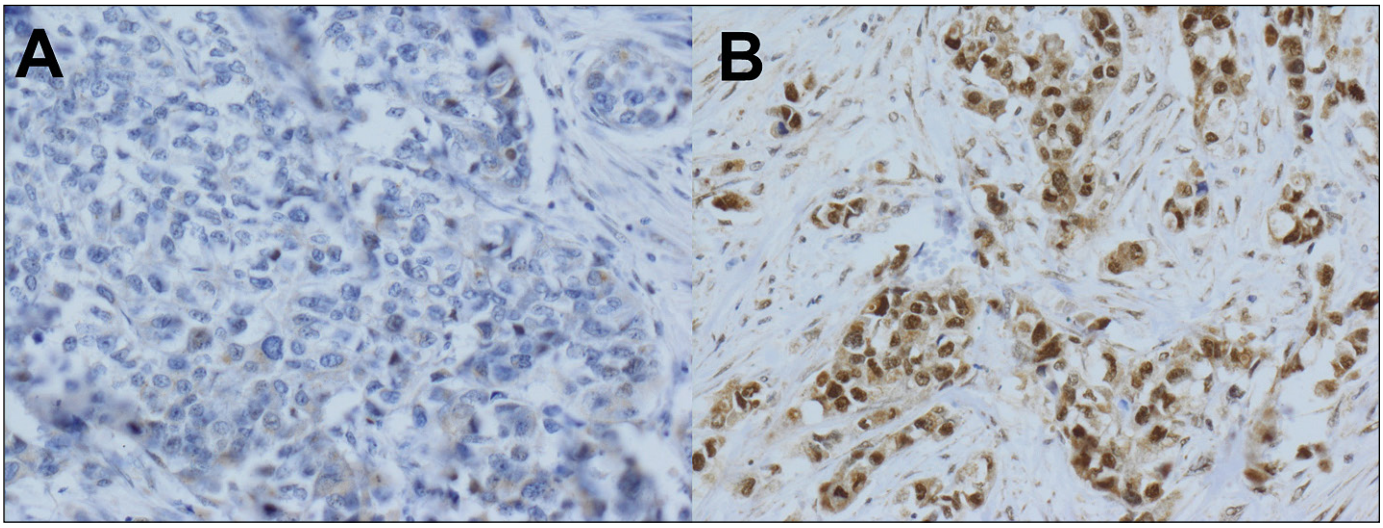

Figure 1. *Microscopic images of negative* **(A)** *and positive* **(B)** *ERCC1 expression.*

whereas in ERCC1-negative tumours 5-year OS was 0.230 (95% CI: 0.140–0.379). Nevertheless, log-rank analysis revealed that survival curves were statistically indifferent in both expression categories (Figure 2).

Univariate and multivariate Cox regression analyses were employed to estimate the risk of death according to ERCC1 expression category and clinical-pathological characteristics. As summarized in Table 2, the risk of all-cause death was not significantly affected by ERCC1 expression category. Age, T-stage, node metastasis and angioinvasion were confirmed to affect HR for death. Such correlations were not found for sex, histopathological grade, ECOG, smoking status, and the presence of at least one comorbidity.

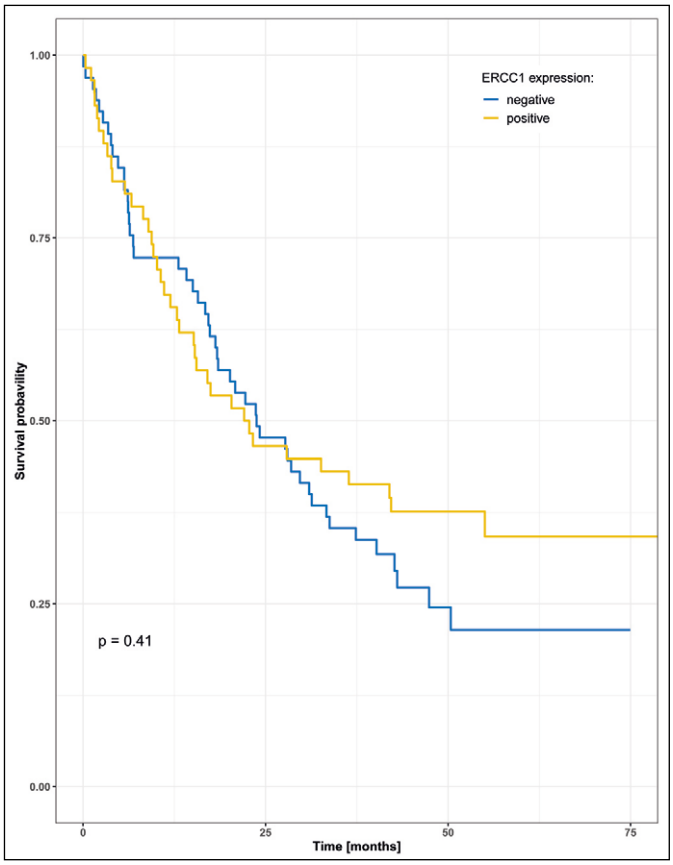

Figure 2. *Survival curves in ERCC1 positive (yellow) and negative (blue) patients.*

DISCUSSION

Since the publication of the first report confirming prognostic significance of ERCC1 in lung adenocarcinoma, it has been evaluated in numerous cancer types in the context of prognosis and response to therapy [15]. ERCC1 has been extensively studied as a biomarker for chemotherapy response in patients with advanced bladder cancer. In general, negative expression of this protein was correlated with better survival, which was summarized in meta-analyses involving 13 studies and 1425 patients [16]. Nevertheless, literature data concerning the role of ERCC1 in patients undergoing radical cystectomy is limited, and in some cases incoherent. Thus, we aimed to evaluate the prognostic significance of this marker protein in a cohort of patients following radical surgical extirpation.

In our analysis positive expression of ERCC1 was noted in approximately 46% of patients, which is in agreement with other publications, where positive expression ranged from 24–76% as summarized by Urun et al. [16]. In most of the papers, expression of ERCC1 was established immunohistochemically (IHC). Nevertheless, the authors utilized various quantification approaches, as well as different threshold values to discriminate positive and negative expression categories. In our analysis, we employed median ERCC1 expression as a cut-off value, similar to other research groups [17–20]. However, other authors considered an H score larger than or equal to 1 [21] or 2 [22, 23], which, together with the various antibody clones used for IHC staining, may be responsible for the discordant study results.

We found no statistically significant correlation between ERCC1 expression categories and the analyzed clinical or pathological characteristics, as summarized in Table 1. Similarly, we could not establish a statistically significant influence of ERCC1 expression on overall survival. Nevertheless, the risk of all-cause death was insignificantly lower in case of positive expression of the studied protein. These observations are in line with the report by Klatte and el. [21], who showed that the risk of BC-specific death or recurrence is reduced in terms of positive expression of ERCC1 in surgical specimens following radical cystectomy. In a group of 432 patients, positive expression was found in 71% of individuals. Patients with ERCC1-positive expression had a significantly better five-year disease-free survival (DFS) than those with ERCC1-negative expression, 62% to 49%, and cancer-specific survival (CSS), 70% to 59%, respectively. In the ERCC1-positive group, the risk of BC recurrence and death due to BC was 30% lower. Patients undergoing radical cystectomy with ERCC1-positive expression had better survival values than those with negative expression. As a consequence, the authors also reported that ERCC1 could be utilized as an independent prognostic factor. Similar results were observed by Sakano who [17] suggested that, in the group of patients with BC undergoing a combined trimodality approach, disease-specific survival might be predicted

Table 2. *Summary of statistical analysis*

Characteristic	Univariate			Multivariate		
	HR[1]	95% CI[1]	p-value	HR[1]	95% CI[1]	p-value
ERCC1 expression category						
ERCC1 negative	–	–		–	–	
ERCC1 positive	0.83	0.54, 1.28	0.4	1.02	0.60, 1.73	>0.9
Patient age						
Below 65	–	–		–	–	
65 and above	1.63	1.05, 2.54	0.030	1.66	0.96, 2.87	0.072
Sex						
Male	–	–		–	–	
Female	0.85	0.46, 1.56	0.6	0.85	0.39, 1.86	0.7
T-stage						
T1-T2	–	–		–	–	
T3-T4	2.95	1.66, 5.26	<0.001	1.49	0.67, 3.27	0.3
N-stage						
N0	–	–		–	–	
N+	2.47	1.59, 3.84	<0.001	1.36	0.78, 2.38	0.3
Grade						
Low-grade	–	–		–	–	
High-grade	1.11	0.51, 2.42	0.8	0.90	0.32, 2.59	0.9
Angioinvasion						
Absent	–	–		–	–	
Present	2.31	1.42, 3.76	<0.001	2.39	1.21, 4.71	0.012
ECOG performance						
0	–	–		–	–	
1 and above	1.24	0.80, 1.91	0.3	0.80	0.44, 1.47	0.5
Comorbidities						
0	–	–		–	–	
1 and above	1.22	0.78, 1.90	0.4	1.55	0.85, 2.85	0.2
Smoking status						
Non-smoker	–	–		–	–	
Smoker	1.06	0.67, 1.66	0.8	1.04	0.60, 1.82	0.9

[1]HR – hazard ratio; CI – confidence interval ; ERCC1 – excision repair cross-complementation 1 protein; ECOG – Eastern Cooperative Oncology Group

by the expression of ERCC1 and X-ray ray cross complementing protein (XRCC1). Positive expression of these molecules was connected with better disease-specific survival rates but further research is needed to confirm these results. Nevertheless, in our analysis, the prognostic value of ERCC1 could not be confirmed in a multivariate Cox-regression analysis, which can partially be explained by the substantial impact of other analyzed clinical and pathological characteristic, such as angioinvasion T- and N-stage. Analyzing the previous studies, one can find contradicting information about predicting the prognostic role of ERCC1 in the treatment of advanced bladder cancer. In 2018, Eldehna [24] conducted a descriptive study involving 80 patients with muscle-invasive bladder cancer (stages T2–T4a) who received platinum-based chemotherapy. The results of their research showed a significant relationship between a platinum-based treatment response and the ERCC1 expression in bladder cancer tissue samples ($p = 0.013$). It was an indicative association between negative immuno-expression and a more favorable outcome but no difference between the ERCC1 expression and mean overall survival or progression-free survival in different immune-expression levels in patients was apparent. Therefore, ERCC1-positive tumors were associated with better prognosis in cases without chemotherapies. However, in cases with chemotherapies, ERCC1-negative tumors were associated with a better outcome. The most probable explanation for the above scenario seems to be related to the function of this gene, which appears crucial in the DNA damage repair ability of the cell. The above DNA repair, related to the ERCC1 activity, appears, however, detrimental for patients treated with chemotherapy. Unfortunately, we did not assess ERCC1 expression in the neoadjuvant setting.

Patients included in our study did not receive neoadjuvant chemotherapy prior to radical cystectomy, an approach commonly utilized according to current guidelines. Thus, it would be interesting to analyze prognostic values of ERCC1 expression

in a neo-adjuvant setting. What is more, it is important to note that our analysis was performed retrospectively in a single institution, which may be regarded as the study limitation. Thus, further prospective studies are needed to corroborate our findings.

CONCLUSIONS

We found no statistically significant correlation between ERCC1 expression categories and analyzed clinical or pathological characteristics. Similarly, we could not establish a statistically significant influence of ERCC1 expression on overall survival. However, in line with previous observations, the risk of all-cause death was insignificantly lower in the case of positive expression of the studied protein.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE – the study was performed with ethical approval and patients' consent

CONSENT FOR PUBLICATION – agreed

AVAILABILITY OF SUPPORTING DATA – data are available in the Deparment

COMPETING INTERESTS NO COMPETING INTERESTS – nothing to disclose

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AUTHORS' CONTRIBUTIONS

Mateusz Obarzanowski: writing, methodology
Antoni Domagała: methodology
Jarosław Jaskulski: methodology
Janusz Kopczyński: methodology
Paweł Macek: methodology
Stanisław Góźdź: supervision
Maciej Salagierski: supervision, methodology

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