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Original article

Decreased PLK1 expression denotes therapy resistance and unfavourable disease-free survival in rectal cancer patients receiving neoadjuvant chemoradiotherapy

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ABSTRACT

Aim: Polo-like kinase 1 (Plk1) plays a key role in mitotic cell division and DNA damage repair. It has been observed that either up-regulated or down-regulated Plk1 could induce mitotic defects that results in aneuploidy and tumorigenesis, probably depending on the context. Few previous reports have associated Plk1 expression with prognosis and response to radiotherapy in rectal carcinomas. The aim of this study is to investigate the prognostic impact of Plk1 expression and its role in predicting response to neoadjuvant cheomoradiotherapy in rectal cancer.

Methods and results: Immunohistochemical analysis of Plk1 expression was performed in the pre-treatment tumour specimens from 75 rectal cancer patients. We analysed the assocation between Plk1 expression and clinicopathological parameters, pathologic response and outcome. Opposed to previous reports on this issue, low expression of Plk1 was significantly associated with a high grade of differentiation ($P = 0.0007$) and higher rate of distant metastasis ($P = 0.014$). More importantly, decreased levels of Plk1 were associated with absence of response after neoadjuvant therapy ($P = 0.049$). Moreover, low Plk1 expression emerged as an unfavourable prognostic factor for disease-free survival in the non-responder group of patients ($P = 0.037$).

Conclusions: Decreased Plk1 expression was associated with poor pathologic response and worse disease-free survival in rectal cancer patients receiving neoadjuvant chemoradiotherapy, suggesting Plk1 as a clinically relevant marker to predict chemoradiotherapy response and outcome.

1. Introduction

Neoadjuvant treatment is the standard of care for locally advanced rectal adenocarcinoma. Preoperative chemoradiotherapy (CRT) and subsequent radical resection have shown to improve outcome in these patients [1]. A complete pathologic response is only observed in approximately 10% to 30% of patients, whereas nearly 40% of patients show poor or no response to preoperative CRT [2].

The primary cytotoxic lesion induced by therapeutic radiotherapy is DNA double-strand breaks (DSBs) [3]. Current research has focused on molecular markers involved in the resistance toward anticancer therapy including key regulators for the mitotic progression and DNA damage repair pathways [4–7]. One of these factors is the serine/threonine kinase Polo-like kinase 1 (Plk1), which is a key for cell division and mitotic progression and DNA damage repair [8,9]. It is overexpressed in many types of human cancers and many of these studies have demonstrated that Plk1 overexpression correlates with tumour progression and patient outcome [10]. However, there is other evidence supporting the role of Plk1

Abbreviations: CRT, chemoradiotherapy; DSB, double-strand break; FFPE, formalin-fixed paraffin-embedded; MRI, magnetic resonance imaging; MTA, microtubule-targeting agent; Plk1, Polo-like kinase 1; TMA, tissue microarray.

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as tumour suppressor [11,12]. Consequently, the reduction of Plk1 may also induce mitotic defects that lead to aneuploidy and tumorigenesis.

The aim of this study is to assess the association of Plk1 expression in biopsies of previously treated locally advanced rectal adenocarcinoma and to evaluate the relation of Plk1 with pathological response to preoperative CRT and outcome.

2. Materials and methods

2.1. Patients and treatment

The records of 91 consecutive patients with clinical stage II or III rectal adenocarcinoma of the usual type, who underwent standardized preoperative CRT followed by surgical resection of the rectum with total mesorectal excision, from December 2006 to January 2014, were reviewed. Only patients with free surgical margins were enrolled in this study. For the aim of the present study mucinous adenocarcinomas were specifically excluded, for they were very few to draw any conclusion and they show a worse prognosis. Diagnosis was confirmed with an endoscopic biopsy in all the cases and patients were staged with magnetic resonance imaging (MRI) and endorectal ultrasound. All patients received 28 sessions of radiation therapy (45 Gy on pelvic area and 50.4 Gy on the tumour bed), concomitantly with fluoropyrimidines-based chemotherapy. Of the 91 eligible patients, we only enrolled those who had enough tissue to perform immunohistochemical analysis (n = 75). The follow-up for this cohort of patients ranged from 4 to 85 months with a median of 20 months.

This study was reviewed and approved by the Institutional Review Board at the University Hospital Fundación Jimenez Diaz and all the patients gave written consent for inclusion. The study is in accordance to Spanish regulations regarding personal data protection.

Table 1
Clinicopathological characteristics of the patients.

Baseline clinical characteristics (*N* = 75)		*N* (%)
Age	<60	14 (19)
	>60	61 (81)
Gender	Male	46 (61)
	Female	29 (39)
ECOG Performance status	0	41 (55)
	1	32 (43)
	2	2 (3)
Neoadjuvant chemotherapy	RT + 5-FU	61 (81)
	RT + 5-FU + oxaliplatin	14 (19)
Grade differentiation	Low	59 (79)
	High	16 (21)
Stage	II	4 (5)
	III	71 (95)
Pathological response	Responder	36 (48)
	Non-responder	39 (52)
T Downstaging	No	29 (39)
	Yes	39 (52)
	N/A	7 (9)
N Downstaging	No	20 (27)
	Yes	48 (64)
	N/A	7 (9)
Status	Death	7 (9)
	Alive with disease	8 (11)
	Alive without disease	59 (79)
	Lost of follow up	1 (1)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; RT, radiotherapy; N/A, not available.

2.2. Histopathological grading of tumour response to therapy

All the specimens of rectal resection after neoadjuvant therapy followed a standardized protocol for tissue sampling. The specimens were fixed with formaldehyde for 72 h and the whole tumour area was embedded in paraffin and serial sections were cut from each block and stained with hematoxylin and eosin. When only mucin pools remained, cytokeratin staining was performed to rule out persistence of isolated epithelial malignant cells.

The regression grade was evaluated in the area with the least response to treatment. Histological assessment of tumour regression was performed by two gastrointestinal-experienced pathologists and cases with discordant results were reviewed cojointly to reach consensus. In unsolved cases, a third pathologist with expertise in gastrointestinal pathology reviewed the case to assign a definite grade. All the pathologists involved in the study were blinded to the outcome of the patients.

Tumor regression was estimated according to recommendations settled by the College of American Pathologists [13], as follows: grade 0 (complete response: absence of tumour cells); grade 1 (moderate response: predominance of fibrosis with isolated tumour cells); grade 2 (minimal response: tumour nests outgrown by fibrosis); and grade 3 (poor response: minimal or no tumour kill). T- and N-downstaging were also assessed.

2.3. Tissue microarray preparation

Formalin-fixed paraffin-embedded (FFPE) tissue samples obtained from diagnostic biopsies before preoperative CRT were used for Tissue Microarray (TMA) construction.

Representative tumour regions were identified by two pathologists (MJFA and FM) on hematoxylin and eosin-stained tissue sections. After pathologists' review, TMAs were assembled from triplicate 0.6 mm cores of FFPE biopsy tumour samples using a TMA workstation MTA-1 (Beecher Instruments, Sun Prairie, WI, USA). We chose to perform a TMA because the material was scarce and we intended to be conservative with it in case it might be needed in the future for further analysis or therapy selection on clinical grounds.

2.4. Immunohistochemistry

Immunohistochemistal staining of pre-treated biopsies was performed on a Dako Autostainer (Dako, Denmark) using anti-Plk1 (1:10; Cell Signaling, Danvers, MA, USA). Human normal testis was used as positive control for Plk1. Results were interpreted by two expert pathologists and the consistency of the assessment between them was over 90%. In cases of disagreement, the expression levels were determined by consensus using a multiheaded microscope.

For the aims of the present study we employed a quickscore system for immunohistochemistry evaluation, considering both the intensity of the staining (in three groups weak, moderate or strong) and the percentage of stained cells. Plk1 was found in the nucleus of tumour cells. With this method we obtained values ranging from 0 to 300. Then we employed tertile values to define the best cut-off point, which was estimated in 30 (meaning 10% of cells with strong staining, 15% of cells with moderate staining or 30% of cells with weak staining). With these criteria we divided the cases in two groups (low and high expression).