Journal of Oncology Research and Treatments

Jaafar, et al., J Oncol Res Treat 2021, S1: 003

Research Article Open Access

Receptor-Interacting Serine/Threonine-Protein-Kinase-2 as a Potential Prognostic Factor in Colorectal Cancer

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Abstract

Introduction: Receptor-interacting-serine/threonine-protein-kinase-2(RIPK2) is an important mediator in different pathways in the immune and inflammatory response system. RIPK2 was also shown to play different roles in different cancer types, however, in Colorectal Cancer(CRC); its role is not well established. This study aims at identifying the role of RIPK2 in CRC progression and survival.

Materials and methods: Data of patients and mRNA proteins' expression level of genes associated with CRC (RIPK2, TNF, TRAF1, TRAF7, KLF6, Il6, Il8, VEGFA, MKI67, TP53, NFKB, NFKB2, BCL2, XIAP, and RELA) were downloaded from PrognoScan online public database. Patients were divided between low and high RIPK2 expression and different CRC characteristics were studied between the 2 groups. Survival curves were evaluated using Kaplan-Meier estimators. Pearson correlation was used to study the correlation between RIPK2 and the other factors. Statistical analysis was carried out using SPSS version 25.0. Human protein atlas was also used for the relation between RIPK2 expression in CRC tissues and survival. Differences were considered statistically significant at $p<0.05$.

Results: A total of 520 patients were downloaded from the PrognoScan, RIPK2 was found to correlate with MKI67, TRAF1, KLF6, TNF, Il6, Il8, VEGFA, NFKB2, BCL2, and RELA. High expression of RIPK2 was associated with high expression of VEGFA ($p<0.01$) and increased mortality ($p<0.01$).

Discussion: In this study, RIPK2 is shown to be a potential prognostic factor in CRC, and however, more studies are needed to assess its potential as a marker for targeted therapy.

Keywords: Receptor-interacting-serine; Inflammatory response; Therapy; Cancer

Introduction

The role of inflammation in promoting cancer cell progression is currently a well-known phenomenon [1,2]. Cancer tissues show signs of inflammation such as the presence of immune cells in the tissue, presence of specific chemokine, and angiogenesis [3]. Chronic inflammation causes tissue damage, which induces cell proliferation and tissue repair and as a consequence tumour development [4-6].

Receptor-interacting-serine/threonine-protein-kinase-2(RIPK2) is an important mediator required in different pathways in the immune and inflammatory response system and was found to be involved in different cancers [7,8]. It is highly expressed in Head and Neck Squamous Cell Carcinoma(HNSCC), and in glioma, it was reported to promote cell proliferation and prevents apoptosis [9,10]. In breast cancer, mainly in Triple-Negative Breast Cancer (TNBC), it was shown to impact patient overall survival, increase recurrence, protect cells from apoptosis induced by chemotherapy and enhance cell proliferation by activating Nuclear-Factor-Kappa-B(NFKB) [11-13]. RIP2 expression correlated with tumour size, metastasis, overall staging, progression-free survival, and Body Mass Index(BMI) of patients with breast cancer, and RIPK2 polymorphism was also involved in the development of bladder cancer [14,15].

Colorectal Carcinoma(CRC) is known to be a potential complication of Inflammatory Bowel Disease(IBD), where it was found to be 60% higher than in the general population [16]. Strong evidence suggests that inflammation is a key element in the etiology of CRC mediated by NFKB [17]. According to Jaafar et al. RIPK2 is associated with the NFKB pathway and seems to have a role in IBD and colitis-associated CRC where the level of expression of RIPK2 was significantly higher in the colonic mucosa of patients with ulcerative colitis compared to controls [18]. In mice, a deficiency in RIPK2 can cause dysbiosis which is a microbial imbalance in the colon, which in turn predisposes mice to communicable colitis and colitis-associated CRC [19]. In a recent study on 4 patients with CRC, RIPK2 was shown to be up regulated in rectal cancer in comparison to normal adjacent mucosa as identified by the ChIP-Seq procedure [20]. Moreover, RIPK2 expression was reported to be associated with the expression of proto-oncogenic proteins including proliferation-MARKER-KI67(MKI67), Tumor-Protein-P53(TP53), and VASCULAR-ENDOTHELIAL-GROWTH-FACTOR-A(VEGFA) [14,15]. These proteins also play a role in the survival and prognosis of CRC patient where high MKI67 expression is correlated with decreased overall survival and disease-free survival, TP53 expression was found to be significantly associated with poor survival, and VEGF expression was

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Received April 08, 2021; **Accepted** April 22, 2021; **Published** April 29, 2021

Citation: Jaafar RF, Ibrahim Z, Ataya K, Hassanieh J, Ard N, et al. (2021) Receptor-Interacting Serine/Threonine-Protein-Kinase-2 as a Potential Prognostic Factor in Colorectal Cancer. J Oncol Res Treat S1: 003.

associated with decreased survival, higher grade, presence of lymph node metastasis, depth of invasion and overall stage [21-26]. This study aims at identifying the role of RIPK2 on CRC patients' prognosis and survival and its association the different pro survival proteins involved in CRC, and presenting RIPK2 as a potential prognostic factor and a therapeutic target.

Materials and Methods

mRNA proteins' expression in CRC were downloaded from PrognoScan online public database [27]. mRNA expression level of genes associated with CRC tumorigenesis included RIPK2 and defined proto-oncogenic proteins MKI67, TP53, VEGF in addition to Tumour-Necrosis-Factor(TNF), TNF-Receptor-Associated-Factor-1(TRAF1), TRAF7, Kruppel-Like-Factor-6(KLF6), Interleukin-6(Il6), Interleukin-8(Il8), NFKB, NFKB2, B-cell lymphoma-2(Bcl2), X-linked-Inhibitor-Of-Apoptosis-Protein(XIAP), and v-Rel-Reticuloendotheliosis-Viral-Oncogene-Homolog-A(RELA) were analysed. Data available included patients' age, gender, follow-up time, CRC site, histological markers including grade, TNM staging where T stands for tumour depth of invasion, N for lymph node involvement, and M for metastasis, and overall stage [28]. Data were downloaded and entered into Statistical Package for the Social Sciences(SPSS) version 25.0 for analysis.

Pearson correlation was used in each dataset separately to study the correlation between RIPK2 and all the other proteins. Datasets were combined, and the median expression of identified proteins was used to divide each dataset into two cohorts, low and high expression, to study the association between low and high expression of RIPK2 and different CRC characteristics. Survival curves were evaluated using the Kaplan-Meier estimators. Differences were considered statistically significant at $p<0.05$. Results from the human protein atlas assessing the role of was RIPK2 in CRC tissues on survival were also extracted and analysed as described by Tran et al. [29].

Results

A total of 4 databases with a total of 520 patients were obtained from the PrognoScan database and detailed shown in Table 1.

RIPK2 expression is associated with tumour site and grade

Comparing patients based on RIPK2 expression, 273(52.5%)

Dataset	Cohort	Contributor	Year	N	Array type	Age(mean ± SD)
GSE12945	Berlin	Staub	2009	62	HG-U133A	64.45 ± 11.78
GSE17536	MCC	Smith	2009	177	HG-U133_Plus_2	65.48 ± 13.08
GSE1433	Melbourne	Jorissen	2010	226	HG-U133_Plus_2	66.03 ± 13.01
GSE17537	VMC	Smith	2009	55	HG-U133_Plus_2	62.31 ± 14.35

Table 1: CRC prognoscan datasets characteristics.

Variable	Low expression of RIPK2 (n=273)	High expression of RIPK2 (n=247)	P
Age (mean ± SD)	65.54 ± 12.40	64.96 ± 13.77	0.61
Sex			
Male	146(53.4%)	130(52.6%)	0.85
Female	127(46.6%)	117(47.4%)	
Site			
Colon	13(37.1%)	16(59.3%)	0.08
Rectum	22(62.9%)	11(40.7%)	
Tumor grade			
1	11(7.6%)	6(4.6%)	
2	109(75.2%)	88(67.7%)	0.09
3	25(17.2%)	36(27.7%)	
T			
2	9(25.7%)	7(25.9%)	
3	25(71.4%)	17(62.9%)	0.41
4	1(2.8%)	3(11.1%)	
N			
0	22(62.8%)	14(51.8%)	
1	6(17.1%)	8(29.6%)	0.5
2	7(20.0%)	5(18.5%)	
M			
0	30(88.2%)	26(96.3%)	0.25
1	4(11.8%)	1(3.7%)	
Stage			
I	51(18.7%)	31(12.5%)	
II	100(36.6%)	89(36.0%)	
III	90(32.9%)	98(39.7%)	0.19
IV	32(11.7%)	29(11.7%)	

Table 2: Patients' characteristics according to RIPK2 expression level.

patients were found to have low expression and 247(47.5%) had high expression (Table 2). The mean age was 65.54+12.40 and 64.96+13.77 respectively. Higher RIPK2 expression was observed in 16(59.2%) patients with colon cancer and 11(40.7%) patients with rectal cancer (p=0.08). Higher expression of RIPK2 was also associated with an increased proportion of grade 3 tumours 36(27.7%) compared to lower expression 25(17.2%) (p=0.09). There was no statistically significant association between RIPK2 expression and lymph node involvement, metastasis, and overall stage (Table 2).

Effect of RIPK2 on the survival of CRC patients

Patients with higher RIPK2 mRNA expression had a significantly higher mortality rate 94(38.06%) in comparison to those with relatively lower expression 61(22.34%) (p<0.01). Survival analysis was also done based on RIPK2 mRNA expression where high expression of RIPK2 was associated with decreased survival in CRC patients (Figure 1).

RIPK2 association with proto-oncogenes

An association between RIPK2 and other proto-oncogenic proteins' mRNA expression was done (Table 3). RIPK2 is associated with high MKI67 and VEGFA mRNA expression, while no significant association was found with TP53 expression. A correlation analysis was also done to understand the relation between the expression of all the basic proteins mentioned above and the RIPK2 expression (Table 4). MKI67, TRAF1, KLF6, Il6, Il8, VEGFA, and RELA were found to positively correlate with RIPK2 and the highest correlation was found between RIPK2 and RELA in the GSE12945 dataset (K=0.43; p<0.01). TNF and BcL2 mRNA expression negatively correlate with RIPK2 in one dataset each with p<0.01. However, for NFKB2, it was shown to positively correlate with RIPK2 in the GSE12945 (k=0.42, p<0.01) but a negative correlation was found in another dataset, the GSE17537 (k=-0.30, p ≤ 0.05). TP53, NFKB, XIAP, and TRAF7 mRNA expression were not significantly correlated with RIPK2 mRNA expression in any of the datasets.

Highlights

- Colorectal cancer is an inflammatory associated cancer
- Inflammatory pathways enhance CRC development
- RIPK2 is highly expressed in high grade CRC tumours

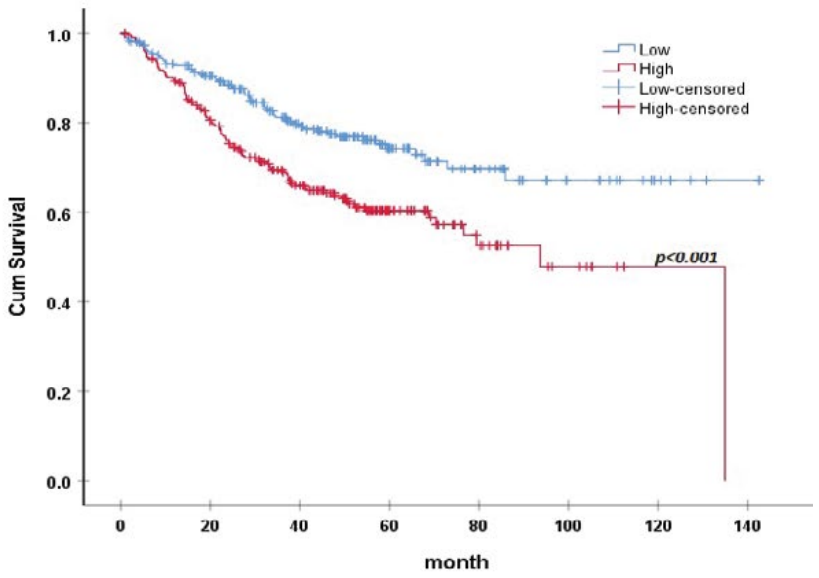

Figure 1: Kaplan-Meier survival plot of patients based on RIPK2 expression.

High expression of gene	Low expression of RIPK2 (n=273)	High expression of RIPK2 (n=247)	P
MKI67	139(47.2%)	141(57.0%)	0.06
TP53	166(60.8%)	155(62.7%)	0.65
VEGFA	116(42.4%)	139(56.2%)	<0.01

Table 3: Association of RIPK2 with proliferation genes expression.

Dataset	MKI67	TRAF1	KLF6	TNF	Il6	Il8	VEGFA	NFKB2	BCL2	RELA
GSE12945	0.16	0.15	0.13	0.09	0.02	0.25*	0.18	0.42**	0.22	0.43**
GSE17536	0.1	-0.03	0.24**	0.12	0.22**	0.41**	0.19*	-0.06	-0.27**	0.12
GSE1433	0.15*	0.16*	0.20**	0	0.25**	0.04	0.20**	0.12	0.01	0.15**
GSE17537	0.40**	0	0.12	-0.35**	0.12	-0.08	0	-0.30*	-0.19	-0.01

Table 4: Correlation between RIPK2 mRNA expression and proteins involved in colorectal cancer.

- High mRNA RIPK2 expression is associated with expression of key proto-oncogenes
- RIPK2 is associated with increased mortality of CRC patients.

Discussion and Conclusion

CRC is the third most common cancer and accounts for 10% of all annually diagnosed cancer, and 9% of cancer-related death [30]. Prognostic factors of CRC include the TNM staging based on which the basis of therapeutic decisions are made, and many potential molecular prognostic markers have been described, unfortunately, most have very limited value in routine clinical practice [31].

Surgery, chemotherapy, radiotherapy, and targeted therapy are all options in metastatic CRC. Target therapy includes anti-angiogenetic factors like bevacizumab, anti-epidermal growth factor receptor-like cetuximab and panitumumab, immune checkpoint inhibitors, anti-BRAF therapy, and HER2-targeted therapy [32]. However, the use of these targeted therapies is limited to factors unique to each patient, for example, the effectiveness of cetuximab is limited to patients with KRAS wild-type tumours, recent studies also showed that the side of the primary tumour affect the outcome of treatment with cetuximab, with the left-sided location being more favourable [33,34]. So discovering other prognostic factors, possible of being targeted by therapy, can improve the outcome of CRC.

We have found that the level of mRNA expression of RIPK2 was found to significantly correlate with several proteins involved in tumorigenesis, and after dividing the patients between high and low expression, those who had higher expression of RIPK2 also had a higher expression of VEGFA ($p<0.01$). RIPK2 was also found to be a prognostic marker in CRC, where higher expression of RIPK2 was associated with worse survival ($p<0.01$).

Exploring the human protein atlas, immunohistochemistry analysis was made for patients with colon cancer and those with rectal cancer, even though no results were significant, but the patients with rectal cancer are the only ones who showed results similar to our analysis, in which patients with higher expression of RIPK2 showed more death. From the data collected from PrognoScan, only 62(11.92%) had information about the site of cancer, so we were not able to do separate analysis by site, but with the results showed in the human protein atlas, we can assume that the majority of our patients had rectal cancer.

Grade and the presence of metastasis are important prognostic factors in CRC, in our analysis based on the PrognoScan data, RIPK2 expression was associated neither with grade ($p=0.09$) nor with the presence of metastasis ($p=0.25$), but it was associated with long term survival ($p<0.01$). However, another limit to our study is that information regarding grade and the presence of metastasis was only present for 275(52.88%) and 61(11.73%) patients respectively. However some studies showed that RIPK2 plays a role in metastasis in different cancer forms, for example in TBC, RIPK2 knockdown decreases migration and lung metastasis, in inflammatory breast cancer, higher RIPK2 activity was correlated with metastasis, and in hepatic cell carcinoma, knockdown of RIPK2 down regulated multiple genes involved in epithelial-mesenchymal transition [12,14,35].

We were also not able to collect data concerning the relation between RIPK2 and recurrence of CRC, it was shown that in TNBC, higher expression of RIPK2 is associated with increased recurrence, hence, further studies should be conducted to determine the role of RIPK2 in metastasis and recurrence in CRC, and to see if it differs depending on the site of the tumour, and its status as a potential target for therapy in metastatic CRC [13].

Authors' Contributions

RJ was responsible for study conception, analysis, write up, revision and final editing; ZI did literature search, data analysis and write up; KA, JH and NA were responsible for data collection; WF was responsible for final manuscript revision and approval. All authors read and approved the final draft.

Availability of Data and Materials

All data related to this paper's conclusion are available and stored by the authors. All data are available from the corresponding author on reasonable request.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgement

We would like to thank Clinical Research Institute at AUBMC that supported in data analysis.

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