EXPRESSION OF HER2 AND PDL-1 IN COLORECTAL CARCINOMA-SINGLE CENTER ANALYSIS

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Abstract: Colorectal cancer as an aggressive malignant neoplasm by incidence has the third place in men after lung and prostate cancer, and the second place in woman after breast cancer. There are near 600.000 deaths every year worldwide associated with colorectal carcinoma. Due to tumor heterogeneity and molecular profile new therapeutic strategies and trials are emerging. HER2, which is more commonly used as a biomarker in breast cancer and gastric cancer, in the last decade has been investigated as potential target treatment in colorectal cancer. After the benefit from anti PD-1 monoclonal antibodies in non-small cell lung carcinoma, melanoma and renal cancer, new clinical trials are evaluating PDL-1 expression and its scoring systems. Our aim in this retrospective-prospective study is to evaluate the immunohistochemical expression of HER2 and PDL-1 and explore the case characteristics. We analyzed 90 cases of metastatic colorectal carcinoma diagnosed at Clinical Hospital Acibadem-Sistina on a tissue microarray with HER2 clone 4B5 and PDL-1 clone SP263. In the HER2 positive group mostly were male patients (72.22%) and patients over 50 years of age (91.67%). Moderately differentiated were mostly HER2-positive tumours with scores of 2+ (78.13%) and 3+(75%). The majority of HER2-positive tumors were localized in the left colon, regardless of the intensity of expression. 12 out of 30 K-RAS/N-RAS positive cases showed HER2 expression, 5 of 9 BRAF positive cases showed HER2 expression and 3 of 5 MMRd cases showed HER2 expression. Male patients were represented by 58.82% and females by 41.18% in the PDL1 positive expression. PDL1 positive tumors with cut off more than 1% were in 70% and cut off more than 10% in 75% of cases were most commonly moderately differentiated and with more than 50% positive tumor cells were most commonly poorly differentiated (66.67%). All 3 cases with more than 50% positive tumor cells were localized in right colon. Four of 26 K-RAS/ N-RAS positive cases showed expression for PDL-1, 3 of 6 BRAF positive cases showed expression for PDL-1 and 2 of 3 MMRd cases showed expression for PDL1. Molecular profiling is mandatory in the treatment of colorectal carcinoma. The official recommendations are to test HER2 expression in all cases with advanced metastatic disease of colorectal carcinoma. PDL-1 in recent years has become a potential target for cancer therapy. Although the role in colorectal cancer is less clear, overexpression of PDL1 is linked with worse prognosis, BRAF mutations and right sided location.
Keywords: colorectal cancer, biomarkers, HER2, PDL-1

1. INTRODUCTION

Colorectal cancer (CRC) is an aggressive malignant neoplasm, with incidence taking second place in women after breast cancer and third place in men after prostate and lung cancer. Adenocarcinoma is the most common histologic subtype. There are 1.2 million new cases of colorectal adenocarcinoma every year in the world. About 600,000 deaths occur each year that are associated with colorectal cancer. In the United States, it accounts for about 10% of the world's malignancy cases and deaths. CRC accounts for approximately 15% of all cancer-related fatalities in the United States, ranking second only to lung cancer. Colorectal cancer incidence is notably high in regions such as Australia, New Zealand, Europe, and increasingly in Japan due to shifts in lifestyle and diet. Conversely, lower rates are observed in South America, India, Africa, and South-Central Asia. People aged 60-70 are most commonly affected, while 20% of cases are people under 50. In early and locally advanced cancers, surgical removal is the best treatment. Current ESMO protocols recommend neoadjuvant therapy for CRC with lymph node metastases when the disease is in Stage III. Adjuvant treatment in high-risk stage II cases is limited and still controversial. In general, the prognosis of patients with CRC is based on the stage of the disease at the time of diagnosis and clinicopathological characteristics. The five-year survival rate for colorectal cancer varies significantly by stage, with approximately 90% survival for stage I, 70% for stage II, 58% for stage III, and less than 15% for stage IV.

Molecular analysis should include an assessment of KRAS and NRAS mutations in codons 12 and 13 of exon two, 59 and 61 of exon three, and 117 and 146 of exon four (https://www.nccn.org). Importantly, not all patients with wild-type KRAS respond to anti-EGFR therapy, and resistance to this treatment remains a critical concern. Anti-EGFR therapy can lead to mutations in KRAS, NRAS, BRAF, and the EGFR ectodomain, which reactivate the MAP kinase pathway despite EGFR inhibition. Identifying additional biomarkers is crucial for selecting patients with wild-type KRAS likely to benefit from anti-EGFR therapy and detecting those who develop resistance, as this personalized approach is often costly. BRAF mutations, which activate the gene in 10% of colorectal cancers, most commonly occur in codon 600 (BRAF V600E), accounting for nearly 90% of these mutations. The prognostic relevance of HER2, frequently seen in sigmoid colon carcinomas, is still debated. However, HER2 is gaining attention as a predictive marker for anti-EGFR therapy. Evidence from multiple studies indicates that acquired amplifications of ERBB2 negatively impact the efficacy of EGFR-targeted treatments and contribute to therapy resistance. Incorporating HER2 assessment into routine practice could provide valuable insights for tailoring treatment strategies and improving patient outcomes.

PD-L1 expression in colorectal cancer is still an incomplete role and studies have shown either a good or bad prognosis. In contrast to some cancers, including melanoma and lung cancer, colorectal cancer generally has a response rate of less than 10% to therapies that target PD-1 or PD-L1 blockade.

2. MATERIALS AND METHODS

We analyzed 90 metastatic cases of colorectal carcinoma diagnosed at Department of Histopathology and cytology at Clinical Hospital Acibadem-Sistina in Skopje. On a tissue microarray immunohistochemical analysis was performed with HER2 clone 4B5 and PDL-1 clone SP263. The expression of HER2 was evaluated as membranous positivity similar to gastric carcinoma, as 1+, 2+ and 3+. PDL-1 was evaluated using cut off scores of percentages of positive membranous staining of tumor cells as >1%, >10% and >50%.

3. RESULTS

HER2 expression

In the HER2 positive expressed and HER2 negative group, male patients (72.22% and 64.81%, respectively), and patients over 50 years of age (91.67% vs 90.74%) were more commonly represented. The mean age of HER2 positive patients was 69.1 ± 12.8 years, HER2 negative patients were at a mean age of 67.4 ± 10.3 years. (Table 1)

Table 1. Demographic characteristics of patients with HER2 expression

variable	HER2		
	n	positive n (%)	negative n (%)
gender			
males	61	26 (72.22)	35 (64.81)
females	29	10 (27.78)	19 (35.19)
age			
n	36		54
mean ± SD	69.1 ± 12.8		67.4 ± 10.3

min- max	34 – 89		35 – 85
Age groups			
≤50	8	3 (8.33)	5 (9.26)
>50	82	33 (91.67)	49 (90.74)

Source: Authors research

Moderately differentiated were mostly HER2-positive tumours with scores of 2+ and 3+ (78.13% and 75%, respectively). The majority of HER2-positive tumors were localized in the left colon, regardless of the intensity of expression (78.13% HER2 2+ vs 75% HER2 3+ tumors). In the right column, the localization of HER2-positive tumours with a score of 2+ was identical (3 tumours individually in the cecum, ascendant and transversum), 1 tumour with a score of 3+ was detected in the ascending colon. In the left colon 30.43% HER2 tumours with a score of 2+ and 66.67% with a score of 3+ were diagnosed in the sigmoid colon, 47.83% HER2 with a score of 2+ and 33.33% with a score of 3+ in the rectum, 21.74% HER2 score 2+ positive tumours were detectable in the descending colon.

Table 2. Grade and localization with HER2 expression

variable	HER 2		
	n	2+ n (%)	3+ n (%)
G			
1	0	0	0
2	28	25 (78.13)	3 (75)
3	8	7 (21.88)	1 (25)
Side			
Right colon	10	9 (28.13)	1 (25)
Left colon	26	23 (71.88)	3 (75)
Right colon			
coecum	3	3 (33.33)	0
c.ascendens	4	3 (33.33)	1 (100)
c.transversum	3	3 (33.33)	0
Left colon			
c.sygmoideum	9	7 (30.43)	2 (66.67)
c.descendens	5	5 (21.74)	0
rectum	12	11 (47.83)	1 (33.33)

Source: Authors research

12 out of 30 K-RAS/N-RAS positive cases showed HER2 expression, 5 of 9 BRAF positive cases showed HER2 expression and 3 of 5 MMRd cases showed HER2 expression.
10 of 30 K-RAS/N-RAS positive cases showed HER2 expression with a score of 2+, 2 tumors had expression with a score of 3+; 5 of 9 BRAF positive cases showed HER2 expression with a score of 2+; 2 of 5 MMRd cases showed HER2 expression with a score of 2+, 1 tumor with a score of 3+. (Table 3)

Table 3.HER2 expression with molecular characteristics

	N=90	**HER2 2+ n=32**	**HER2 3+ n=4**
K-RAS/ N-RAS			
+	30(33.33)	10 (31.25)	2 (50)
-	60(66.67)	22 (68.75)	2 (50)
BRAF			
+	9(10)	5 (15.63)	0
-	81(90)	27 (84.38)	4 (100)
MMR			
d	5(5.56)	2 (6.25)	1 (25)
p	85(94.44)	30 (93.75)	3 (75)

Source: Authors research

PDL-1expression

Male patients were represented by 58.82% and 69.86%, respectively, in the positive expression and negative group, female by 41.18% and 30.14%, respectively, in the PDL1 positive expression and negative group. The mean age of PDL1 positive patients was 68.7 ± 11.9 years, PDL1 negative patients were at mean age of 67.9 ± 11.3 years. (Table 4)

Table 4. Demographic characteristics of patients and PDL-1 expression

variable	PDL 1		
	n	positive n (%)	negative n (%)
gender			
males	61	10 (58.82)	51 (69.86)
females	29	7 (41.18)	22 (30.14)
age			
n	17		73
mean ± SD	68.7 ± 11.9		67.9 ± 11.3
min- max	49 – 85		34 – 89
Age group			
≤50	8	2 (11.76)	6 (8.22)
>50	82	15 (88.24)	67 (91.78)

Source: Authors research

PDL1 positive tumors with cut off more than 1% and more than 10% positive tumor cells were most commonly moderately differentiated (70% and 75%, respectively), with more than 50% positive tumor cells most commonly poorly differentiated (66.67%). Left-sided localization most commonly had PDL1 positive tumors with more than 1% and more than 10% positive tumor cells (90% and 75%, respectively). All 3 cases with more than 50% positive tumor cells were localized in right colon. (Table 5)

Table 5. Grade and localization with PDL1expression

variable	PDL 1			
	n	PDL >1% n (%)	PDL >10% n (%)	PDL >50% n (%)
G				
1	0	0	0	0
2	11	7 (70)	3 (75)	1 (33.33)
3	6	3 (30)	1 (25)	2 (66.67)
Side				
Right colon	5	1 (10)	1 (20)	3 (100)
Left colon	12	9 (90)	3 (80)	0
Right colon				
coecum	2	0	1 (100)	1 (33,33)
c.ascendens	2	0	0	2 (66,67)
c.transversum	1	1 (100)	0	0
Left colon				
c.sygmoideum	3	2 (22.22)	1 (33.33)	0
c.descendens	2	1 (11.11)	1 (33.33)	0
rectum	7	6 (66.67)	1 (33.33)	0

Source: Authors research

Four of 26 K-RAS/ N-RAS positive cases showed expression for PDL-1, 3 of 6 BRAF positive cases showed expression for PDL-1 and 2 of 3 MMRd cases showed expression for PDL1. Two of 26 K-RAS/N-RAS positive cases showed a cut off of PDL-1 positive cells greater than 1%, 2 tumors greater than 10%. 1 of 6 BRAF positive cases showed a cut off of PDL-1 positive cells greater than 1%, 3 tumors greater than 50%, 1 of 3 MMRd tumors presented a cut off of PDL-1 positive cells greater than 1%, 1 tumor greater than 10%. (Table 6)

Table 6. PDL-1 expression cut offs with molecular characteristics

	N=90	PDL >1% n=10	PDL >10% n=4	PDL >50% n=3
K-RAS/ N-RAS				
+	26 (28.89)	2 (20)	2 (50)	0
-	64 (71.11)	8 (80)	2 (50)	3 (100)
BRAF				
+	6 (6.67)	1 (10)	0	3 (100)
-	84 (93.33)	9 (90)	4 (100)	0
MMR				
d	3 (3.33)	1 (10)	1 (25)	0
p	87 (96.67)	9 (90)	3 (75)	3 (100)

Source: Authors research

4. DISCUSSION

Somewhere between 3-4% of colorectal cancer patients may have overexpressed the HER2 gene and its receptor. The frequency of HER2 gene amplification is higher in wild-type KRAS patients, where it is 6-8%. This amplification is also more common in left-sided colon tumors than in right-sided ones. In our study, 4.44% of the tumors were found to be positive for HER2 and all of them were found in the left colon. According to the National Comprehensive Cancer Network, all patients with stage IV metastatic colorectal cancer have to have HER2 biomarker testing. PDL-1 in recent years has become a potential target for cancer therapy. Although the role in colorectal cancer is less clear, overexpression of PDL1 is linked with worse prognosis, BRAF mutations, and right-sided location. Our results showed 3 positive cases with cut-off >50% that were located in the right colon and all had BRAF mutations.

5. CONCLUSION

Molecular features of HER2 and PD-L1 expressing cases are consistent with previously published findings. HER2 amplification or overexpression is usually correlated with poor response to EGFR inhibitors. An important point to mention is that HER2 testing should be performed prior to beginning oncologic therapy with EGFR inhibitors. Moreover, the high expression of PD-L1 in colorectal cancer can provide a survival advantage by inhibiting the immune response.

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