MOLECULAR CHARACTERIZATION OF MICROPAPILLARY VARIANT OF COLORECTAL CARCINOMA. REVIEW OF SIXTY CASES

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Background

Micropapillary carcinoma (MC) is accepted as an aggressive variant of colorectal adenocarcinoma, characterized histologically by small papillary cell clusters surrounded by lacunar spaces (Figure 1A). It was described for the first time in the breast and later in other organs including urinary bladder, lung, ovary and salivary gland. At present, there are a limited number of colorectal MC series reported. Even though some of these present immunohistochemistry (IHC) and molecular results, the information published is not enough to define the molecular profile of this variant, aim of this study.

Materials & Methods

Clinicopathological features of a cohort of 379 patients with primary colorectal cancer were retrospectively reviewed, looking for the presence, quantification and localization of MC pattern respect to the whole tumoral mass. The parameters evaluated in each case included: age, sex, tumor location and size, growing pattern, grade, depth of invasion (pT), vascular and perineural invasion, nodal status (pN) and number of positive lymph nodes. We also assessed the expression of CK7, CK20, CEA, MUC1, EMA, p53 and Miss Match Repair genes (MMR), achieved following an avidin-biotin immunoperoxidase procedure. Likewise, genetic assessment of microsatellite instability (MIN), chromosome 18q status, p53 and KRAS mutation were performed on DNA extracted from formalin-fixed, paraffin-embedded samples.

Results

Table 1 summarizes clinicopathological findings. MC component, involving at least 5% of the tumor volume, was identified in 60 out of 379 cases (15.8%), ranging from 5 to 95% of the tumor. MC cases presented significantly higher frequency of infiltrative pattern and more positive lymph nodes compared with conventional carcinoma (Figure 2). IHC for MUC1 and EMA makes evident the characteristic inside-out staining pattern of the MC component, whereas the rest of the tumor shows luminal staining patterns (Figures 1B, C1 and C2). Also, these component showed more frequently CK7 expression (23.1 to 13.3%), (p=0.548) (Figure 1D). CK20 staining was observed in the majority of MC and conventional carcinomas, and CEA in all of them (Table 2). Table 3 summarizes the molecular results. Statistically significant differences were found between the two groups on the frequency of p53 alterations (accumulation and/or mutation) (p=0.034), MIN (p=0.012), and incidence of RER phenotype (MMR loss and/or MIN) (p=0.011). No association was observed between MC category and KRAS or BRAF mutations.

Categorical variable	% MC >=5% (n= 60)	% MC <5% (n = 319)	*p*-value
Gender			0.669
Male	37 (61.7)	185 (58.0)	
Female	23 (38.3)	134 (42.0)	
Location			0.662
Proximal	24 (40.0)	113 (35.4)	
Distal	36 (60.0)	196 (61.4)	
Unknown	0	10 (3.1)	
Configuration			**0.024**
Ulcerated / Stenosing	42 (70.0)	171 (53.6)	
Polipoid / Exophytic	18 (30.0)	145 (45.5)	
Unknown	0	3 (0.9)	
Histology			0.083
Adenocarcinoma	57 (95.0)	275 (86.2)	
Mucinous adenocarcinoma	3 (5.0)	43 (13.5)	
Grade (WHO)			**0.018**
1	23 (38.3)	171 (53.6)	
2	30 (50.0)	84 (26.3)	
3	7 (11.7)	64 (20.1)	
Extent of invasion (pT)			**<0.001**
pT1	3 (5.0)	18 (5.6)	
pT2	2 (3.3)	51 (16.0)	
pT3	23 (38.3)	157 (49.2)	
pT4	32 (53.3)	93 (29.2)	
Stage			**<0.001**
1	3 (5.0)	59 (18.5)	
2	9 (15.0)	127 (39.8)	
3	38 (63.3)	108 (33.9)	
4	10 (16.7)	25 (7.8)	
Lymph node involvement (pN)			**<0.001**
0	12 (20.0)	192 (60.2)	
1	22 (36.7)	74 (23.2)	
2	26 (43.3)	53 (16.6)	
Growth pattern			**<0.001**
Infiltrative	58 (96.7)	199 (62.4)	
Expansive	2 (3.3)	118 (37.0)	
Unknown	0	2 (0.6)	
Cytologic grading			**0.003**
High-grade	40 (67.0)	145 (45.5)	
Low or intermediate grade	20 (33.0)	172 (53.9)	
Unknown	0	1 (0.3)	
Peritoneal invasion			0.001
Present	30 (50.0)	92 (27.6)	
Absent	30 (50.0)	231 (72.4)	
Metastasis (pM)			**0.048**
Present	10 (16.7)	25 (7.8)	
Absent	50 (83.3)	294 (92.2)	
Thin-walled vessel invasion			**<0.001**
Present	51 (85.5)	120 (37.6)	
Absent	9 (15.0)	198 (62.1)	
Unknown	0	1 (0.3)	
Venous vessel invasion			**<0.001**
Present	28 (46.7)	61 (19.1)	
Absent	32 (53.3)	258 (80.9)	
Perineural invasion			**<0.001**
Present	18 (30.0)	37 (11.6)	
Absent	42 (70.0)	282 (88.4)	
Crohn-like lymphoid reaction			0.057
Present	15 (25.0)	122 (38.2)	
Absent	45 (75.0)	197 (61.8)	
TIL			0.535
Present	6 (10.0)	44 (13.8)	
Absent	54 (90.0)	274 (85.9)	
Unknown	0	1 (0.3)	
Adenomas			0.417
Present	18 (30.0)	78 (24.5)	
Absent	41 (68.3)	236 (74.0)	
Unknown	1 (1.7)	5 (1.6)	

Numerical variable	% MC >=5 (n= 60) Mean ± SD	% MC <5 (n = 319) Mean ± SD	*p*-value
Age (years)	65.8 ± 11.7	69.3 ± 12.0	**0.023**
Tumor size (mm ∅ maximum)	37.5 ± 12.3	43.8 ± 21.0	**0.032**
Solid carcinoma (%)	11.5 ± 15.2	8.5 ± 19.2	**<0.001**
Mucinous carcinoma (%)	7.3 ± 15.3	13.1 ± 26.4	0.867
Cribriform structures (%)	9.3 ± 18.5	8.1 ± 15.7	0.909
Metastatic nodes (n)	3.5 ± 3.3	1.9 ± 3.9	**<0.001**

TABLE 1: Univariate analysis to correlate clinicopathological features with MC.

IHC expression	% MC >=5% (n= 26)	% MC <5% (n = 30)	*p*-value
CK7			0.549
Positive	6 (23.1)	4 (13.3)	
Negative	20 (76.9)	26 (86.6)	
CK20			0.899
Positiva	25 (96.1)	28 (93.3)	
Negative	1 (3.9)	2 (6.7)	
CEA			1
Positive	26 (100)	30 (100)	
Negative	0 (0)	0 (0)	

TABLE 2: Univariate analysis to correlate immunohistochemitry expression with MC.

Molecular variable	% MC >=5 (n= 60)	% MC <5 (n = 319)	*p*-value
P53 accumulation			0.119
Present	39 (65.0)	169 (53.0)	
Absent	21 (35)	149 (46.7)	
Unknown	0	1 (0.3)	
P53 mutation			0.056
Present	45 (75.0)	193 (60.5)	
Absent	15 (25)	121 (37.9)	
Unknown	0	5 (1.6)	
P53 altered (mut. and/or accum.)			**0.034**
Present	48 (80.0)	208 (65.2)	
Absent	12 (20.0)	108 (33.9)	
Unknown	0	3 (0.9)	
17p loss			0.448
Present	27 (45.0)	141 (44.2)	
Absent	26 (43.3)	106 (33.2)	
Unknown	7 (1.2)	72 (22.6)	
18q loss			0.541
Present	41 (68.3)	179 (56.1)	
Absent	17 (28.3)	93 (29.2)	
Unknown	2 (3.3)	47 (14.7)	
MSI (NCI panel)			0.024
MSS	51 (85.0)	240 (75.2)	
MSI-L	3 (5.0)	21 (6.6)	
MSI-H	1 (1.7)	39 (12.2)	
Unknown	5 (8.3)	19 (6.0)	
MSI (NCI + 17p and 18q panel)			**0.012**
MSS	49 (81.7)	215 (67.4)	
MSI-L	4 (6.7)	47 (14.7)	
MSI-H	1 (1.6)	34 (10.7)	
Unknown	6 (10.0)	23 (7.2)	
MMR loss of expression			0.063
Present	1 (1.7)	27 (8.5)	
Absent	59 (98.3)	282 (88.4)	
Unknown	0	10 (3.1)	
RER phenotype (MMR loss and/or MIN)			**0.011**
Present	1 (1.7)	40 (12.5)	
Absent	54 (90.0)	251 (78.7)	
Unknown	5 (8.3)	28 (8.8)	
Kras mutation			0.464
Present	27 (45.0)	121 (37.9)	
Absent	30 (50.0)	172 (53.9)	
Unknown	3 (5.0)	26 (8.2)	
Braf V600E mutation			0.143
Present	9 (15.0)	26 (8.2)	
Absent	46 (76.7)	257 (80.6)	
Unknown	5 (8.3)	36 (11.3)	

TABLE 3: Univariate analysis to correlate molecular features with MC.

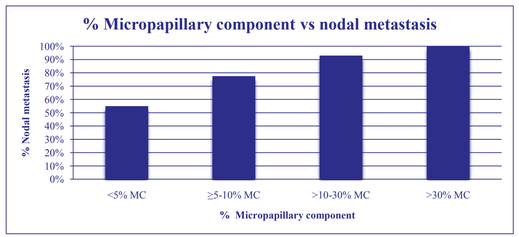

FIGURE 2 : Distribution micropapillary component vs metastasis.

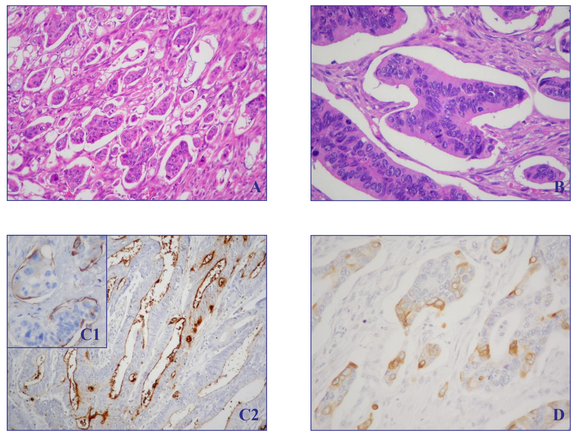

Figure 1. Histological findings of micropapillary pattern. A, characteristic cell clusters surrounded by lacunar spaces and dense fibrous stroma (H&E, 200x). B, tumor nest with reverse cell polarity (H&E, 400x). C1, Immunohistochemistry for MUC-1 showing characteristic "inside-out" staining pattern in the MC component (MUC-1, 400x). C2, the rest of the tumor shows luminal staining pattern (MUC-1, 200x). D, MC cells showing CK7 expression (H&E, 400x).

Conclusions

- Regarding the pathological and molecular parameters, colorectal MC appears more aggressive than conventional colorectal adenocarcinoma.
- Colorectal MC is associated with higher tumor stages, frequent vascular and perineural invasion and nodal metastasis (80 vs 39,8%).
- MUC-1 at the interface tumor cells-stroma could contribute to construct the characteristic morphological features of MC and its invasive potential, interacting directly with the stroma.
- It is important to know the more frequent expression of CK7 (23.1%) in colorectal MC when evaluating micropapillary metastatic tumor of unknown origin.
- Colorectal MC is associated with frequent p53 alterations (80%), lower incidence of MSI and RER phenotype, supporting the chromosomic instability pathway of carcinogenesis as should be expected for the aggressive profile of colorectal MC.

References

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