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Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

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GEDII GRUPO DE ESTUDO DA DOENÇA INFLAMATÓRIA INTESTINAL

Methylation Patterns

In Dysplasia

In Inflammatory Bowel Disease

Patients

Overview

1. Introduction
2. Aim
3. Methods
4. Results
5. Discussion/Conclusion
6. Questions

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Inflammatory bowel disease (**IBD**) with colonic involvement

increases colorectal cancer (CRC) risk.

Colitis-related dysplasia differs from conventional dysplasia,

and both may occur in IBD patients.

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Colitis-related dysplasia	**Conventional dysplasia**
Inflammation > Dysplasia > Carcinoma	Adenoma > Carcinoma
Multifocal	Focal
Non adenoma-like	Adenoma-like
TP53 mutation frequent / initiating	*TP53* mutation frequent / late stage
Low rate of *KRAS* and *APC* mutations	High rate of *APC* (initiating) and *KRAS* mutations

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Dysplasia in IBD patients

Colitis-related dysplasia

↓

Endoscopic resection: Polypoid +/- Flat lesions

Colectomy: ?Flat lesions

Conventional dysplasia

↓

Managed as general population

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Good marker specific for the type of dysplasia (Colitis-related / Conventional)

?

2. Aim

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Some data favors the importance of **abnormal DNA methylation** in colitis-related carcinogenesis.

Hypothesis: Lesions that evolved through different pathways would show different methylation patterns.

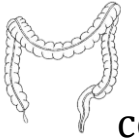
Aim: Identify methylation markers that could later be prospectively evaluated.

3. Methods

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Patients with colonic IBD

↓



Samples of colonic mucosa

Paraffin-embedded biopsies and surgical specimens

→

A: **With** dysplasia/CRC

B: <10cm from dysplasia/CRC, without IBD-colitis

C: >10cm from dysplasia/CRC, **with** inactive IBD-colitis

D: >10cm from dysplasia/CRC, **with** active IBD-colitis

E: >10cm from dysplasia/CRC, without IBD-colitis

Inclusion criteria:

A + (B and/or C and/or D and/or E)

3. Methods

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Patients with colonic IBD

Samples of colonic mucosa

Paraffin-embedded biopsies and surgical specimens

Without dysplasia/CRC (**B, C, D, E**)

With dysplasia/CRC (**A**)

Colitis-related (Cases)

Random biopsies of flat mucosa

Non adenoma-like lesion

Conventional (Controls)

Dysplasia in non-IBD affected area

Adenoma-like lesion

3. Methods

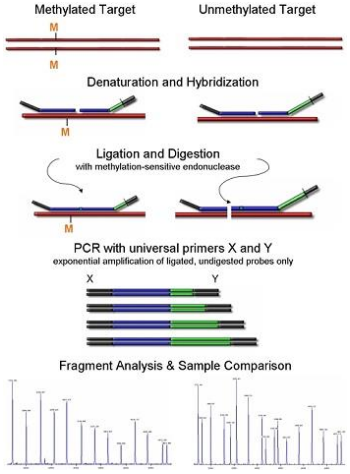
Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Patients with colonic IBD

Samples of colonic mucosa

- Without dysplasia/CRC (**B, C, D, E**)
- With dysplasia/CRC (**A**)
  - Colitis-related (Cases)
  - Conventional (Controls)

(MS-MLPA)



Methylation patterns of CpG islands in the promoter regions of 67 genes implicated in CRC carcinogenesis

RUNX3. CDH1. SOCS1. ESR. CDKN2A. APC. SFRP1. MLH1. TGFβ. BMP. WNT. IGF2. RARB. ESR1. CHFR. CDH13. WT1. GATA5. WIF1. TIMP3. MSH6. MSH3. CRABP1. TP73. RARB. CDH13. PAX5. WT1. THBS1. TP53. SFRP1. WIF1. APAF1. BCL2. …

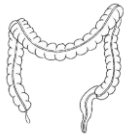
4. Results

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Patients with colonic IBD

N = 35

29 UC, 6 CD



Samples

N = 91

2-6 samples per patient

No dysplasia/CRC (**B, C, D, E**)

N = 56

Dysplasia/CRC (**A**)

N = 35

Colitis-related (Cases)

N = 9

Conventional (Controls)

N = 26

Methylation patterns of promotors of 67 genes involved in CRC carcinogenesis

N=56/91

No adenoma-like and non-adenoma-like lesions in the same patient.

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Patient's characteristics		Colitis-related dysplasia/CRC (index lesion)		Significance (X^2/Exact/T tests)
		No	Yes	
Gender	Male	17	5	p= 0.698
	Female	9	4	
Diagnosis	Crohn's Disease	4	2	p= 0.635
	Ulcerative Colitis	22	7	
Age at IBD diagnosis		46.73 +/- 13.81 (22-65)	29.22 +/- 16.08 (14-64)	**p= 0.003**
Age at dysplasia/cancer diagnosis		58.50 +/- 12.92 (31-84)	47.67 +/- 13.30 (30-71)	**p= 0.039**
Active inflammation in the dysplasia/CRC area	No	22	4	**p= 0.030**
	Yes	4	5	

Patients with colitis-related dysplasia/CRC were **younger** at IBD diagnosis and dysplasia/CRC diagnosis.

Colitis-related dysplasia/CRC were more common in areas with **active inflammation**.

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

67 genes involved in CRC carcinogenesis

- **23 without** promoters' hypermethylation
- **44 with** promoters' hypermethylation

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

44 genes with promoters' hipermethylation

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More common

in dysplastic/CRC (A)

vs not-dysplastic samples (B.C.D.E)

(p< 0.001 – p= 0.043)

IGF2. RARB. ESR1. CHFR.

WT1. GATA5. WIF1

15

More common

in high-grade dysplasia/CRC (subset of A)

vs LG-dysplastic/not-dysplastic samples (B.C.D.E)

(p= 0.001 – p= 0.038)

MSH6. MSH3. MGMT. RUNX3. CDKN2A.

IGF2. RARB. ESR1. CADM1. TIMP3.

PAX5. PAX6. WT1. THBS1. SFRP1

15

More common

in active-IBD (D)

vs inactive or non-affected IBD (B.C.E)

(p= 0.001 – p= 0.038)

MSH6. MSH3. RUNX3. CRABP1. TP73.

RARB. CDH13. PAX5. WT1. THBS1.

TP53. SFRP1. WIF1. APAF1. BCL2

4. Results

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

44 genes with promoters' hypermethylation

Aim: Identify methylation markers (for dysplasia pathways in IBD patients) that could later be prospectively evaluated.

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(*MSH6, TIMP3*)

More common in colitis-related dysplastic/CRC (subset of A)

than conventional dysplastic/CRC samples (subset of A)
(p= 0.002 and p= 0.012)

MSH6

Multivariate analysis (p= 0.029)

Gene	Methylation	Inflammation		Significance (X^2/Exact)	Dysplasia		Significance (X^2/ Exact)	HGD/cancer		Significance level (X^2/ Exact)	IBD-related dysplasia/cancer		Significance (X^2/ Exact)
		No	Yes		No	Yes		No	Yes		No	Yes	
MSH6	No	64	15	**p=0.007**	44	35	p=0.712	75	4	p=0.010	30	5	p=0.002
	Yes	5	7		6	6		8	4		1	5	
MSH3	No	68	19	p=0.042	49	38	p=0.323	82	5	p=0.002	29	9	p=1.000
	Yes	1	3		1	3		1	3		2	1	
MGMT	No	67	21	p=0.569	50	38	p=0.088	82	6	p=0.020	28	10	p=0.564
	Yes	2	1		0	3		1	2		3	0	
RUNX3	No	51	17	**p=0.020**	37	31	p=0.105	63	5	p=0.001	23	8	p=1.000
	Yes	0	3		0	3		0	3		2	1	
NEUROG1	No	41	12	p=0.076	29	24	p=0.451	49	4	p=0.189	19	5	p=0.395
	Yes	10	8		8	10		14	4		6	4	
CDKN2A	No	50	18	p=0.189	37	31	p=0.105	62	6	p=0.032	23	8	p=1.000
	Yes	1	2		0	3		1	2		2	1	
IGF2	No	36	13	p=0.647	32	17	p=0.001	47	2	p=0.009	13	4	p=1.000
	Yes	15	7		5	17		16	6		12	5	
CRABP1	No	50	16	p=0.020	35	31	p=0.665	60	6	p=0.094	23	8	p=1.000
	Yes	1	4		2	3		3	2		2	1	
CACNA1G	No	49	18	p=0.314	36	31	p=0.344	61	6	p=0.060	23	8	p=1.000
	Yes	2	2		1	3		2	2		2	1	

(...)

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

DNA methylation occurs in genes implicated in CRC carcinogenesis

Inflammation alters DNA methylation in IBD mucosa

Methylation of *MSH6* promoter region is significantly associated to colitis-related dysplasia/CRC, irrespective of the grade (multivariate analysis).

15% of colitis-related CRC carcinogenesis is MSI, some cases due to MSH6[1]

??Due to methylation??

[1]Harpaz and Polydorides (2010).

Methylation Patterns In Dysplasia In Inflammatory Bowel Disease Patients

Methylation of MSH6 promoter region

may contribute to the classification of dysplastic lesions in IBD patients

and eventually identify patients at risk for multifocal dysplastic/cancer lesions.

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THANK YOU!

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