EZH2 expression in colorectal (CRC) cancer: single nucleotide polymorphism (SNP) characterization and correlation with clinico-pathological and molecular parameters

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Background: EZH2 is an epigenetic factor, essential for stem cell self-renewal. *EZH2* rs3757441 SNP is associated with outcome in metastatic CRC patients treated with FOLFIRI+/-bevacizumab.
Material and Methods: We are investigating the correlation between EZH2 expression and other clinico-pathological and molecular parameters in 129 primary CRC samples. EZH2 expression was evaluated by immunohistochemistry, *KRAS* codon 12-13-61 and *BRAF* V600E mutations were screened by pyrosequencing analysis, and *EZH2* SNP variants were identified by real-time PCR. Studied parameters were correlated with EZH2 expression by Fisher's exact test, chi-square test, t-test and ANOVA.
Results: Characteristics of the specimens are: stage I/II/III/IV, 5/54/47/23; grading 1/2/3, 0/81/48; right colon/left colon/rectum, 56/49/24; mucinous histology, 40; *KRAS* wt/mut, 74/55; *BRAF* wt/mut, 115/14; rs3757441 genotype (119 samples genotyped so far) CC/CT or TT, 5/114. Staining intensity is stronger in tumors harbouring the rs3757441-CC genotype (p=0.023). EZH2 expression does not correlate with other studied parameters: however, the percentage of EZH2-positive cells is significantly higher in mucinous than in non-mucinous tumors (p=0.045).
Conclusions: rs3757441-CC genotype is associated with stronger EZH2 immunoreactivity, confirming a role for the SNP in controlling EZH2 expression. Moreover, mucinous CRCs show higher EZH2 expression, which may contribute to a more aggressive tumor behavior. EZH2 and EZH2-related pathways deserve further investigation as putative prognostic indicators and candidate therapeutic targets in CRC.

Background

- ✓ Cancer stem cells (CSCs) are the seeds of tumour formation, progression and resistance to treatment[1]
- ✓ EZH2 is essential for CSC self-renewal in several solid tumors and is involved in tumour angiogenesis[2]
- ✓ EZH2 expression increases with stage in colorectal cancer (CRC)[3]
- ✓ High EZH2 mRNA expression predicts shorter OS in CRC patients[4]
- ✓ EZH2 single nucleotide polymorphisms (SNPs) are associated with lung cancer risk[5]
- ✓ EZH2 is the catalytic subunit of PRC2, a multimeric complex belonging to Polycomb group genes (PcGs)[6]
- ✓ PcGs are epigenetic effectors essential for stem cell self-renewal and gene silencing[7]
- ✓ EZH2 catalyzes histone H3 lysine 27 trimethylation and triggers gene silencing[8]
- ✓ EZH2 mediates E-cadherin silencing and cancer cell invasion[2]
- ✓ With few exceptions (*KRAS* and *BRAF* V600E mutations) molecular prognostic biomarkers in metastatic CRC (mCRC) are lacking[9]
- ✓ Moreover, no reliable predictive factors of benefit have been identified for cytotoxics and bevacizumab[10]
- ✓ mCRC patients harbouring the C/C genotype for the *EZH2* 626-394C>T (rs3757441) SNP have worse outcome compared to C/T or T/T patients when treated with FOLFIRI +/- bevacizumab[11,12]
- ✓ C/C genotype for the *EZH2* 626-394C>T SNP is associated with higher EZH2 mRNA expression compared to C/T or T/T in peripheral lymphocytes[11]

Objectives

Primary:

✓ Correlate EZH2 expression evaluated by immunohistochemistry (IHC) on CRC primary tumor samples with *EZH2* 626-394C>T SNP genotype

Secondary:

✓ Correlate EZH2 expression evaluated by immunohistochemistry (IHC) on CRC primary tumor samples with

- ➔ tumor stage, grading and mucinous histology
- ➔ *KRAS* codon 12, 13 and 61 and *BRAF* V600E mutations

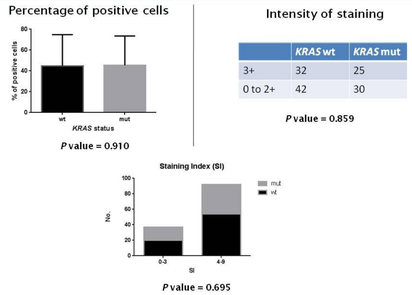
Methods

- ✓129 paraffin-embedded primary tumor samples of CRC patients operated at Azienda Ospedaliero-Universitaria Pisana were collected
- ✓IHC for EZH2 was conducted as described by Fluge et al, *BJC* 2009[13]
- ✓DNA was extracted from normal colonic mucosa of surgical specimens
- ✓Real-time PCR was used to genotype patients for *EZH2* 626-394C>T SNP
- ✓To evaluate *KRAS* and *BRAF* status, sequencing analyses were conducted as described in Masi et al, *Lancet Oncol* 2010[14]
- ✓Fisher's exact test, chi-square test, t-test or ANOVA were used as appropriate to evaluate association between EZH2 expression and pathologic or molecular features
- ✓EZH2 expression was categorized as:
  - ➔ percentage of positive cells (0-100%)
  - ➔ staining intensity (0, no staining; 1, weak; 2, moderate; and 3, strong)
  - ➔ Staining Index (SI, as categorised by Fluge et al.[13]): calculated as the product (0-9) of positive cells (0, 0%; 1, <10%; 2, 10-50%; and 3, >50%) and staining intensity

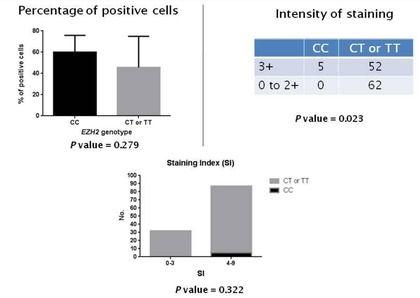
Samples characteristics

Characteristics	No.=129	%
Stage, *I / II / III / IV*	5/54/47/23	4/42/36/18
Grade, *1 / 2 / 3*	0/81/48	0/63/37
Mucinous histology, *yes / no*	40/89	31/69
Site, *Right colon / Left colon / Rectum*	56/49/24	43/37/18
KRAS status, *wild-type – mutant*	74/55	57/43
KRAS mutation (codon), *12 / 13 / 61*	37/15/3	67/27/6
BRAF status, *wild-type / mutant*	115/14	89/11
EZH2 SNP status, *CC / CT / TT*	5/51/63	4/43/53
EZH2 IHC: staining intensity, *neg / 1+ / 2+ / 3+*	19/15/32/63	15/11/25/49
EZH2 IHC: positive cells, *0% / <10% / 10-50% / >50%*	20/1/52/56	16/1/40/43
Staining Index, *0-3 / 4-9*	37/92	29/71

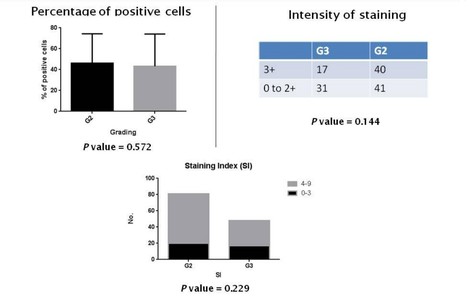
EZH2 Expression and *KRAS* Status



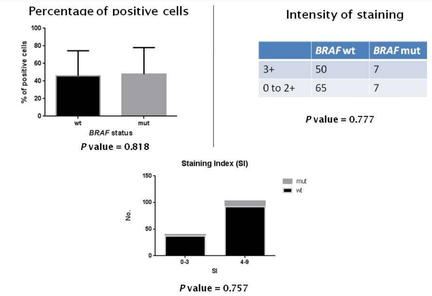
EZH2 Expression and 626-394C>T Genotype



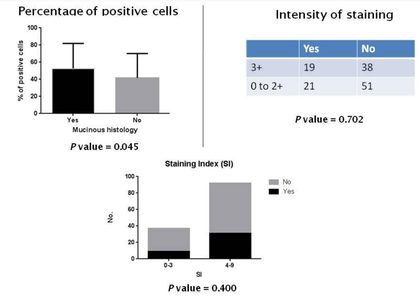
EZH2 Expression and Tumor Grade



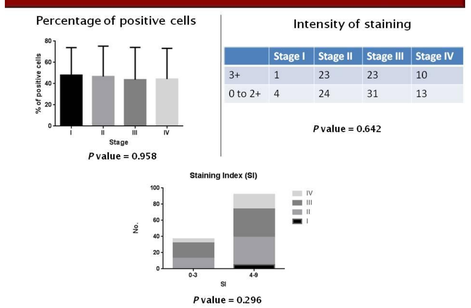
EZH2 Expression and *BRAF* Status



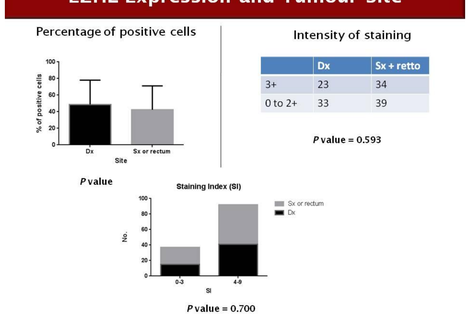
EZH2 Expression and Mucinous histology



EZH2 Expression and Tumor Stage



EZH2 Expression and Tumour Site



Conclusions

- ✓ Primary CRC samples harbouring the C/C genotype for *EZH2* 626-394C>T SNP show significantly stronger EZH2 positivity at IHC compared to C/T or T/T cases
- ✓ There is a trend toward higher percentage of EZH2 positive cells and higher staining index in primary CRC samples harbouring the C/C genotype for *EZH2* 626-394C>T SNP compared to C/T or T/T cases
- ✓ The percentage of EZH2-positive cells is significantly higher in mucinous than in non-mucinous tumors
- ✓ Other investigated pathological features (i.e. tumor stage, grade and site) and molecular alterations (*KRAS* and *BRAF* mutations) show no association with EZH2 expression in our series
- ✓ EZH2 and EZH2-related pathways deserve further investigation as putative prognostic indicators and candidate therapeutic targets in CRC

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