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DELETIONS IN METASTATIC COLORECTAL CANCER WITH CHROMOTHRIPSIS

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Aim: **In our previously reported study, we found a correlation between DNA massive fragmentation and increased progression free survival (PFS) in metastatic colorectal cancer (mCRC), but not overall survival. The aim of this study is to find overlapping deleted genome regions in selected mCRC patients with chromothripsis and detect possible cause of increased PFS, and find new genes or combinations, involved in colorectal cancer oncogenesis.** ***Materials and Methods:*** **10 mCRC patients with chromothripsis receiving 5-fluorouracil, oxaliplatin, leucovorin (FOLFOX) first-line palliative chemotherapy between August, 2011 and October, 2012 were selected for this study. Microarray analysis was performed using the Infinium HumanOmniExpress-12 v1.0 formalin-fixed paraffin-embedded (FFPE) BeadChip kit (Illumina). BeadChip was scaned on HiScan (Illumina). Analysis was performed by GenomeStudio software (Illumina) and R version 3.1.2. Copy number variation and breakpoints on the chromosomes were analyzed using the DNA copy package.** ***Results:*** **Eight deleted tumor suppressor genes (*ROBO2, CADM2, FAT4, PCDH10, PCDH18, CDH18, TSG1, CTNNA3*) and four deleted oncogenes (*CDH12, GPM6A, ADAM29, COL11A1*) were identified in more than half of patients. In 70% patients' deletion in *COL11A1* was detected. Deletion of *MIR1269*, *MIR4465, MIR1261* and *MIR4490* in patients with longer time to progression was observed. Four patients (40%) with PFS over 14 months, presented with *NRG3* deletion (oncogene, epidermal growth factor receptor (EGFR) ligand) what could possibly decrease proliferation of cancer cells via decreasing EGFR activation.** ***Conclusions:*** **Multiple chromosomal deletions (*MIR1269, NRG3, ADK*) in mCRC patients with chromothripsis are associated with better response to first line palliative FOLFOX-type chemotherapy and increased PFS.**
Key Words**: metastatic colorectal cancer, chromothripsis, deletion, progression free survival.**

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According to cancer multiple hit hypothesis, cells acquire multiple mutations and chromosomal rearrangements step by step during cancerogenesis. Colorectal cancer (CRC) development often includes chromosomal instability leading to amplifications and deletions of large DNA segments. Copy number variations — deletions and amplifications — such as mutation of *APC, RAS, p53, DCC* are recently described in CRC oncogenesis. The impact of these events on survival and treatment outcome in CRC are majorly unclear.

In 2011, Stephens *et al.* [1] reported chromothripsis that is observed in 2–3% cancers — a massive chromosome fragmentation occurred as a single catastrophic event leading to chromosomal shuttering in one or more chromosomes and consequent random repair .

Chromothripsis can drive the development of cancer through several mechanisms such as deletion of tumor suppressor genes and activation of proto-oncogenes. Simultaneous loss of multiple tumor suppressor genes located on different chromosomes can lead to cancer formation and further progression.

The prevalence, impact on prognosis and metastasis formation of chromothripsis is not clearly known. Incidence of chromothripsis is from 1.3% in multiple myeloma [2], 6.6% in acute myeloid leukemia [3] to 33% in osteosarcoma [1] and 52.6% in metastatic CRC (mCRC) [4]. Chromothripsis has been associated with poor survival rates in aggressive subtype of breast cancer, multiple myeloma and medulloblastoma [2, 5, 6]. The prognostic and predictive role of chromothripsis in CRC is still unclear.

In our previously reported study [4], we found a correlation between DNA massive fragmentation chromothripsis and increased progression free survival (PFS) in mCRC, but not overall survival (OS). In mCRC with chromothripsis, cancer genome harbours multiple deletions that could lead to better response to 5-fluorouracil, oxaliplatin, leucovorin (FOLFOX) type first line treatment and increased survival.

The aim of this study is to find overlapping deleted genome regions in selected patients with chromothripsis and detect possible cause of increased progression free survival. Also, one of our interests is to find new genes or combinations, involved in CRC oncogenesis.

MATERIALS AND METHODS

Study population. 19 mCRC cancer patients who received chemotherapy in Clinic of Oncology of Pauls Stradins Clinical University Hospital (Riga, Latvia) between August, 2011 and October, 2012 were selected in our study, reported previously [4]. The study was performed with approval from the ethics committee of Riga Stradins University and written informed consent was taken from each patient who underwent the study. Tissue samples were previously acquired as part of series of routine diagnostic and pathological analyses at the Hospital.

Ten of 19 previously analyzed patients with mCRS was detected with chromothripsis phenomena. In this

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Abbreviations used: CRC – colorectal cancer; EGFR – epidermal growth factor receptor; FFPE – formalin-fixed paraffin-embedded; FOLFOX – 5-fluorouracil, oxaliplatin, leucovorin; mCRC – metastatic colorectal cancer; OS – overall survival; PFS – progression free survival.

study we analyzed only patients with chromothripsis. Clinical characteristics of 10 patients are shown in Table 1. All ten patients received FOLFOX type first line chemotherapy. One patient received liver and peritoneal metastasis cytoreductive surgery. After progression 8 patients received irinotecan containing second line chemotherapy; two patients underwent best supportive care. Only two patients (20%) received third line therapy. One patient underwent hepatic surgery for colorectal metastases after discontinuation of second line chemotherapy, one patient — salvage transcatheter arterial chemoembolization of CRC liver metastases by irinotecan-eluting microspheres. We obtained data on clinical follow up until December, 2016. The patients had 4 to 48 months of follow up. mPFS in ten patients was 14 months (range 4–26 months).

Genotyping. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples with QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Quality was evaluated using the Illumina FFPE QC kit (Illumina, San Diego, CA, USA) by reverse transcription-polymerase chain reaction. DNA was restored with the Illumina DNA restoration kit (Illumina). Microarray analysis was performed using the Infinium HumanOmniExpress-12 v1.0 FFPE BeadChip kit (Illumina). BeadChip was scaned on HiScan (Illumina). Analysis was performed by GenomeStudio software (Illumina) and R version 3.1.2. (https://www.r-project.org/). Copy number variations and breakpoints on the chromosomes were analyzed using the DNA copy package (http://bioconductor.org/pack-ages/release/bioc/html/DNAcopy.html).

Statistical analysis. OS and progression free survival rates were estimated using the Kaplan — Meier method. The log-rank test was used to calculate any significant difference between the subgroups by univariate analysis. Significance levels were set at $p < 0.05$. All statistical analyses were performed by using MedCalc.

RESULTS

In 10 tumor samples we found multiple chromosomal fragmentations (> 100 breakpoints detected at one chromosome) — chromothripsis. The most affected chromosomes were chromosome 1, chromosome 2 and chromosome 6. We analyzed deleted regions which were overlapping in 5 or more patients (> 50%).

Frequently deleted regions are shown in Table 2. Genes are identified, divided in five groups: 1) genes involved in proliferation or drug resistance, 2) possible tumor suppressor genes, 3) protocadherin/cadherin genes, 4) epigenetic regulators, and 5) genes of unclear function in oncogenesis. Overlapping deletions are seen in Table 3.

Eight deleted tumor suppressor genes (*ROBO2, CADM2, FAT4, PCDH10, PCDH18, CDH18, TSG1, CTNNA3*) and four deleted oncogenes (*CDH12, GPM6A, ADAM29, COL11A1*) were identified in more than half of patients. In 70% patients' deletion in *COL11A1* was detected. Deletion of *MIR1269, MIR4465, MIR1261* and *MIR4490* in patients with longer time to progression was observed. Four patients (40%) with PFS over 14 months, presented with *NRG3* deletion (oncogene) what could possibly decrease proliferation of cancer cells via decreasing epidermal growth factor receptor (EGFR) activation. Longer PFS was associated with deletion of *MIR1269, MIR4465, MIR1261* and *MIR4490.*

DISCUSSION

Eight deleted tumor suppressor genes (*ROBO2, CADM2, FAT4, PCDH10, PCDH18, CDH18, TSG1, CTNNA3*) and four deleted oncogenes (*CDH12, GPM6A, ADAM29, COL11A1*) were identified in more than half of patients. In 70% patients' deletion in *COL11A1* was detected. It is reported previously that *COL11A1* overexpression is associated with proliferation, migration and chemo-resistance to platinum in lung cancer [7]. We can suggest that deletion of this oncogene could be associated with better survival due to slower progression and migration.

In 50% patients' overlapping deletion of *MIR1269A* was seen — overexpression of it promotes metastases and proliferation in hepatocellular carcinoma [8]. In our study we observe deletion of *MIR1269*, as well as *MIR4465, MIR1261* and *MIR4490*, in patients with longer time to progression. It might be beneficial for mCRC patients to harbor deletions of *MIR* genes because of decreased potential of proliferation and formation of metastases.

50% showed *MAD2L1* deletion. Overexpression of *MAD2L1* is reported in platinum resistant testicular germ cell tumors [9].

Four patients (40%) with PFS over 14 months, presented with *NRG3* deletion what could possibly decrease proliferation of cancer cells via decreasing EGFR activation.

In previous publication we reported chromothripsis as a surrogate marker for increased mPFS and possible indicator for better response to oxaliplatin based

Table 1. Clinical characteristics of patients (n = 10)

Patient №	PFS, months	Age	Tumor grade	CEA, ng/ml	Primary tumor	Metastases	KRAS status
21	4	74	2	343,6	Rectum	Liver, lungs	mt
14	6	74	2	19,5	Colon	Liver, peritoneum	nk
3	8	38	2	7,1	Colon	Peritoneum	wt
6	8	68	3	644,4	Colon	Liver, suprarenes, retroperitoneal l/n	mt
9	11	67	3	959,8	Colon	Liver	wt
18	14	63	2	10,2	Colon	Liver	wt
19	14	60	nk	2,4	Rectum	Peritoneum	wt
20	21	70	2	2,6	Colon	Liver	mt
24	24	52	3	0,8	Colon	Peritoneum	wt
8	26	65	2	2,4	Colon	Peritoneum, solitary liver mts	mt

Note: CEA – carcinoembryonic antigen before chemotherapy; mts – metastases; nk – not known; mt – mutant; wt – wild type.

Table 2. Frequently deleted genes in study population (n = 10)

Gene	Description, importance of gene	COSMIC
1. Genes involved in cell proliferation/drug resistance		
COL11A1	*COL11A1* is overexpressed in recurrent non-small cell lung cancer and promotes cell proliferation, migration, invasion and drug resistance [7]	1174/41930
MAD2L1	Possible resistance to chemotherapy [9]	70/32840
ADAM29	The expression of *ADAM29* and its mutations in different domains significantly influenced proliferation, migration and invasion of breast cancer and colorectal cancer cells [11]	510/33364
GPM6A	Identification of *GPM6A* and *GPM6B* as potential new human lymphoid leukemia-associated oncogenes [12]	167/32746
EPHA7	Downregulation or loss of EphA7 mRNA expression was detected in prostate carcinomas [13]	616/34447
ADK	Adenosine kinase gene expression was significantly higher in cancer than in normal-appearing tissue, in line with our previous measurements of adenosine kinase enzyme activities in colorectal tumor samples [14]	81/32840
NRG3	This gene is a member of the neuregulin gene family. This gene family encodes ligands for the transmembrane tyrosine kinase receptors *ERBB3* and *ERBB4* – members of the epidermal growth factor receptor family	458/33190
2. Putative tumor suppressors		
ROBO2	Down-regulation of *ROBO2* expression is detected in prostate cancers [9]	753/42092
CADM2	Aberrant methylation and loss of *CADM2* tumor suppressor expression is associated with human renal cell carcinoma tumor progression. Low *CADM2* expression predicts high recurrence risk of hepatocellular carcinoma patients after hepatectomy [15, 16]	297/41801
FAT4	*FAT4* functions as a tumour suppressor in gastric, breast and colorectal cancer [17, 18, 19]	1907/33867
TSG1	The grade of *TSG* expression was found to be significantly associated with gastric cancer patient survival. *TSG1* deletion was found in prostatic cancer [20, 21]	109/32746
CTNNA3	*CTNNA3* is tumor suppressor gene in hepatocellular carcinoma. Loss of expression in found in multiple tumor types [22, 23]	504/32927
3. Epigenetic regulators		
Mir4275 *MIR1269A*	miR-1269 promotes colorectal cancer metastasis. miR-1269a is upregulated in late-stage CRCs. Promotes proliferation in hepatocellular carcinoma through suppression of *FOXO1* [8, 10]	
MIR2054 *MIR4643* *MIR4490* *LINC02549*		
PRDM9	With histone methyltransferase activity that catalyzes histone H3 lysine 4 trimethylation (*H3K4me3*) during meiotic prophase	701/32967
4. Unclear/probably unrelated		
GABRG1		403/41616
GABRG2		317/41865
COX7B2		46/41646
TECRL		247/41578
UGT2B4		283/32747
GLRA3		204/32748
FAM174A		47/32746
ST8SIA4	A modulator of the adhesive properties of neural cell adhesion molecule (*NCAM1*)	187/32812
PRR16	Encodes Largen – a molecular regulator of mammalian cell size control. Largen is an important link between mRNA translation, mitochondrial functions, and the control of mammalian cell size [24]	163/32724
FTMT		240/32746
KHDRBS2		303/32878
EYS	The protein is expressed in the photoreceptor layer of the retina, and the gene is mutated in autosomal recessive retinitis pigmentosa	424/33464
MANEA		123/32809
FUT9		218/32746
GRIK2		518/33002
SSPO		
SGCZ	*SGCZ* encodes one of the sarcoglycan complex protein's, that is part of the dystrophin-associated glycoprotein complex, which bridges the inner cytoskeleton and the extra-cellular matrix	258/32747
CNTN5	Neuronal membrane protein that functions as a cell adhesion molecule	602/33002
5. Protocadherin/cadherin gene superfamilies		
PCDH10	*PCDH10* inhibits the proliferation, invasion and migration ability of pancreatic cancer cells and is frequently downregulated by promoter methylation in pancreatic cancer cells [25] The epigenetic silencing of *PCDH10* has been identified as an important tumor suppressor gene with key roles in colorectal carcinogenesis, invasion and metastasis as a frequent and early event [26]. The loss of *PCDH10* function promotes not only tumor progression but also liver metastasis. The genetic deletion of *PCDH10* represents an adverse prognostic marker for the survival of patients with colorectal cancer [27]	763/32849
PCDH18	*PCDH18* is frequently inactivated by promoter methylation in colorectal cancer [28]	563/32837
CDH18	Mutation and loss of expression of cadherins have been implicated in the progression of some malignant tumors, suggesting that cadherins may also act as tumor/metastasis suppressor genes. *CDH18* may be functionally linked to CRC development [29, 30]	670/33085
CDH12	Cadherin-12 enhances proliferation in colorectal cancer cells and increases progression. High expression of *CDH12* was associated with tumor invasion depth and predicts poor prognosis. *CDH12* is expected to become a new diagnostic and prognostic marker and a novel target of the treatment of colorectal cancer [31, 32]	560/32970
CDH10	*CDH10* mutation might inactivate the cell adhesion-related functions and could be a feature of gastric and colorectal cancer with MSI-H [33]	799/33400
PCDH15	*PCDH15* is a member of the cadherin superfamily, encode integral membrane proteins that mediate calcium-dependent cell-cell adhesion	1187/33222

Note: COSMIC – Catalogue of Somatic mutations in Cancer.

treatment — chromothripsis was associated with mPFS 14 *vs* 8 months ($p = 0.03$), but association with OS was not detected [4].

We observed that patients with multiple overlapping deletions have increased time to progression. There are two patients in our study group who are exceptional. Patient № 21 presents multiple overlapping deletions but has very short time to progression. This patient was diagnosed with bulky metastases in liver and lungs, discontinued fist line FOLFOX treatment due to poor performance status and toxicity and progressed in 4 months. Second exceptional patient

Table 3. Overlapping deletions

PFS, months	**4**	**6**	**8**	**8**	**11**	**14**	**14**	**21**	**24**	**26**
Patient number	21	14	6	3	9	18	19	20	24	8
ROBO2										
ROBO1										
CADM2 GBE1										
PROS1 NSUN3 DHFRL1 EPHA6										
PRKAA2 C1orf168										
NEGR1										
LRRIQ3 TNNI3K FPGT-TNNI3K										
ELTD1										
COL11A1										
MIR4275										
GABRG1 GABRA2 GANRA4 COX7B2										
ADGRL3										
TECRL										
miR-1269										
UGT2B4										
MAD2										
FAT4										
PCDH10										
PCDH18										
DCLK2 LRBA										
FSTL5										
GALNTL6										
ADAM29										
GPM6A										
CDH18										
CDH12 PRDM9 CDH10										
FAM174A ST8SIA4 SLCO4C1										
PRR16 FTMT										
KHDRBS2										
EYS										
LINC02549										
BAI3										
MiR-4643 LOC101929083										
EPHA7 TSG1 MANEA FUT9										
GRIK2										
mir-4465										
SSPO										
SGCZ										
PCDH15 MTRNR2L5										
CTNNA3										
ADK										
NRG3										
MiR-4490 miR-1261										
CNTN5										

№ 8 had 26 months remission period but presented only one overlapping deletion (*SSPO*, gene with unknown function). Such a long PFS was observed due to fact that he underwent resection of primary tumor in colon and resection of metastases in peritoneum and liver (maximum cytoreductive surgery) before start of palliative chemotherapy. It is known that treatment strategy — surgery, chemotherapy intensity, targeted treatment, as well as patient related factors, such as performance status, comorbidities, burden of metastatic disease and tumor molecular characteristics, impacts time to progression and OS in mCRC patients.

For further research it is important to have more homogenous patient group with similar clinical findings and treatment strategy.

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