Sequence Variation in Mature MicroRNA-608 and benefit from neo-adjuvant treatment in locally advanced rectal cancer patients.

Sclafani, F., Chau, I., Cunningham, D., Lampis, A., Hahne, J. C., Ghidini, M., ... Valeri, N. (2016). Sequence Variation in Mature MicroRNA-608 and benefit from neo-adjuvant treatment in locally advanced rectal cancer patients. Carcinogenesis. DOI: 10.1093/carcin/bgw073

Published in:
Carcinogenesis

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
© The Author 2016. Published by Oxford University Press. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Download date:04. Jul. 2017
ORIGINAL MANUSCRIPT

Sequence variation in mature microRNA-608 and benefit from neo-adjuvant treatment in locally advanced rectal cancer patients

Francesco Sclafani1, Ian Chau1, David Cunningham1, Andrea Lampis2, Jens Claus Hahne2, Michele Ghidini2, Hazel Lote1,2, Domenico Zito5, Josep Tabernero3, Bengt Glimelius4, Andres Cervantes5, Ruwaida Begum1, David Gonzalez De Castro1, Sanna Hulkki Wilson1, Clare Peckitt1, Zakaria Eltahir1, Andrew Wotherspoon1, Diana Tait1, Gina Brown1, Jacqueline Oates1, Chiara Braconi1,6 and Nicola Valeri1,2,*

1Department of Medicine, The Royal Marsden NHS Foundation Trust, Surrey SM2 5PT, UK, 2Department of Molecular Pathology, The Institute of Cancer Research, Surrey SM2 NG, UK, 3Department of Medical Oncology, Vall d’Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona 08035, Spain, 4Department of Immunology, Genetics and Pathology, Experimental and Clinical Oncology, University of Uppsala, Uppsala 78751 85, Sweden, 5Department of Haematology and Medical Oncology, Biomedical Research Institute INCLIVA, University of Valencia, Valencia 46010, Spain and 6Department of Cancer Therapeutics, The Institute of Cancer Research, Surrey SM2 SNG, UK

*To whom correspondence should be addressed. Centre for Molecular Pathology, The Institute of Cancer Research & The Royal Marsden NHS Foundation Trust, 15 Cotswold Road, Sutton, Surrey SM2 SNG, UK. Tel: +44 0208 915 6634; Fax: +44 0208 643 9414; Email: nicola.valeri@icr.ac.uk

Abstract

Single nucleotide polymorphisms (SNPs) in microRNA genes have been associated with colorectal cancer (CRC) risk, survival and response to treatment. Conflicting results are available on the association between rs4919510, a SNP in mature miR-608 and clinical outcome in CRC. Here, we analyzed the association between rs4919510 and benefit from perioperative treatment in a randomised phase II trial of neoadjuvant Capecitabine and Oxaliplatin (CAPOX) followed by chemo-radiotherapy, surgery and adjuvant CAPOX ± Cetuximab in high-risk locally advanced rectal cancer (LARC).

A total of 155/164 (94.5%) patients were assessable. 95 (61.3%) were homozygous for CC, 55 (35.5%) heterozygous (CG) and 5 (3.2%) homozygous for GG. Median follow-up was 64.9 months. In the CAPOX arm the 5-year progression-free survival (PFS) and overall survival (OS) rates were 54.6% and 60.7% for CC and 82.0% and 82.1% for CG/GG, respectively (HR PFS 0.13, 95% CI: 0.12–0.83, \(P = 0.02\); HR OS 0.38, 95% CI: 0.14–1.01, \(P = 0.05\)). In the CAPOX-C arm PFS and OS were 73.2 and 82.2%, respectively for CC carriers and 64.6 and 73.1% for CG/GG carriers (HR PFS 1.38, 95% CI: 0.61–3.13, \(P = 0.44\); HR OS 1.34, 95% CI: 0.52–3.48, \(P = 0.55\)). An interaction was found between study treatment and rs4919510 genotype for both PFS (\(P = 0.02\)) and OS (\(P = 0.07\)). This is the first study investigating rs4919510 in LARC. The CC genotype appeared to be associated with worse prognosis compared to the CG/GG genotype in patients treated with chemotherapy and chemo-radiotherapy alone. Addition of Cetuximab to chemotherapy and chemo-radiotherapy in CC carriers appeared to improve clinical outcome.
Management of locally advanced rectal cancer (LARC) is largely based on a number of clinical–radiological factors identified on baseline staging. However, it is clear that patients with similar risk factors at baseline can have different outcomes and may require tailored treatment approaches and surveillance strategies (1). Unfortunately, no predictive/prognostic biomarkers are currently available in this setting to allow personalised approaches based on tumor biological aggressiveness or initial response to neoadjuvant treatment.

MicroRNAs are short, non-coding RNA sequences that regulate gene expression by targeting hundreds of mRNAs (2). A number of key cellular processes including proliferation, differentiation and response to anticancer treatments are influenced by this regulatory mechanism (3).

Single nucleotide polymorphisms (SNPs) in microRNA genes have been increasingly analysed for their functional implications. A single base pair change in the nucleoside sequence can have functional consequences. Importantly, SNP genotyping and molecular analyses are currently available in this setting to allow personalised treatment approaches based on tumor biological aggressiveness or initial response to neoadjuvant treatment.

In our study, we report the first study of the role of rs4919510 in a prospective randomised phase II trial (EXPERT-C) of neo-adjuvant Capectabine and Oxaliplatin (CAPOX) followed by chemoradiotherapy (CRT), surgery and adjuvant CAPOX compared with Cetuximab (CAPOX-C arm).

The study was approved by local ethics committees and institutional review boards and written informed consent was obtained from each patient before study entry including consent for future research (ISRCTN registration: 99828560).

SNP selection, genotyping and molecular analyses

Only patients who had tumor tissue available for genomic analysis were eligible for this study. We selected SNPs in microRNA genes and their binding sites previously associated with response to treatment in CRC (7,12) to test for a potential association with response to neoadjuvant chemotherapy and Cetuximab. Results on the association between let-7 complementary site 6 (LC56 KRAS variant) and outcome in the EXPERT-C trial have previously been reported (13).

DNA was extracted from formalin-fixed paraffin-embedded tumor tissue (FFPE) from pre-treatment biopsy and/or post-treatment resection specimens using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) and from peripheral blood mononuclear cells (PBMC) with QIAamp DNA Blood Mini Kit on QIAcube (Qiagen) as we previously described (14). Samples were genotyped using the Taqman assay (Life Technologies, Carlsbad, CA) for rs4919510 in mir-608. Cases, negative controls, and duplicate samples were processed in a random order, with 10% duplicates to test both inter- and intra-plate concordance. All parties involved in genotyping were blinded to the clinical data. Both inter- and intra-plate duplicates were 100% concordant. Mutational analyses of KRAS (exons 2–4), NRAS (exons 2–4), BRAF (codon 600) and TP53 (exons 4–9) were performed centrally on genomic DNA as we previously described (15,16).

Statistical analysis

The Chi-square test was used to assess whether the mir-608 genotypes in the study population were in Hardy–Weinberg equilibrium. Progression-free survival (PFS) was defined as the time between randomization and tumor progression or death. Patients alive and without evidence of tumor progression at the time of the analysis were censored at last follow-up.Overall survival (OS) was defined as the time between randomization and death (or censored at last follow-up for patients who were alive at the time of the analysis). The Kaplan–Meier method was used to calculate survival estimates, and comparison of the treatment arms was carried out using a log-rank analysis. Hazard ratios and 95% confidence intervals were obtained from Cox regression. An interaction term between treatment arm and mir-608 genotype was included in the Cox regression to test for a significant interaction. Multivariate Cox regression was used to assess whether a significant interaction remained significant after addition of prognostic variables. Prognostic variables included sex, World Health Organization (WHO) performance status at baseline (0 versus ≥1), baseline T stage (T4 versus other), TNM stage (stage II versus stage III) baseline miR-EMVI, RAS status (wild-type versus mutant) and TP53 status (wild-type versus mutant) were included in the multivariate models using forward selection if P value in univariate analyses was <0.1.

Results

Genotyping of the rs4919510 locus was performed on DNA extracted from pre (n = 113) and post (n = 122) neo-adjuvant treatment FFPE biopsies in 155 out of 164 eligible patients enrolled in the EXPERT-C trial: this cohort represents 94.5% and is representative of the trial population as we have previously shown (15) (tumor blocks were not available in the remaining cases). The same analysis was carried out in 105 pre-treatment matching peripheral blood samples (64% of the trial population; blood was not available in the remaining cases).

Eighty-one cases had both pre and post treatment biopsies: in this group the concordance rate between pre and post-chemotherapy genotyping was 98.7%. One discordant case showed GC genotype in the pretreatment biopsy and GG genotype in the resection specimen. In this case it is possible that neoadjuvant treatment might have altered the genotype but given that all
the survival outcomes were calculated from randomization, only the pretreatment genotype was used in our analysis. DNA isolated from tumor tissues has been widely used for pharmacogenomic studies; the concordance rate between frequencies of SNPs in tumors and their matching bloods appears quite high with discordance rate lower than 1.5% (17).

In order to rule out any bias due to somatic alterations (such as loss of heterozygosity) that might have affected genotyping in FFPE versus bloods, we compared bloods and tumor tissues in the 101 patients for whom both materials were available and we observed 100% concordance. Interestingly the case with discordant findings between pre and post-treatment tumor samples showed concordance of results between pre-treatment tumor sample and blood, thus supporting our decision to use the pretreatment genotype for analysis. Based on this observation we performed all our survival analyses on data obtained from FFPE material in order to increase the statistical power of the study.

Sixty patients (38.7%) were found to carry the polymorphic variant and these were evenly distributed between the two treatment groups (Table 1). The frequency of the miR-608 genotypes did not deviate from the Hardy–Weinberg equilibrium (P = 0.379) and no significant association was observed between genotype and baseline clinical–pathological characteristics or mutations in the KRAS, NRAS, BRAF, PIK3CA and TP53 gene. (Table 2).

After a median follow-up of 64.9 months (95% CI: 62.8–67.2), no statistically significant differences in PFS [63.5% (95% CI: 53.7–73.3) versus 72.9% (95% CI: 61.5–84.3) at 5 years; HR 0.67 (95% CI: 0.37–1.21) P = 0.18] and OS [71.0% (95% CI: 61.8–80.2) versus 77.6% (95% CI: 66.8–88.4) at 5 years, HR 0.66 (95% CI: 0.34–1.26), P = 0.208] were observed in the overall population between patients homozygous for the C allele and those carrying the G allele.

In the CAPOX arm, patients with the CC genotype had worse 5-year PFS [54.6% (95% CI: 40.5–68.7) versus 82.0% (95% CI: 67.7–96.3) HR 0.13 (95% CI: 0.12–0.83) P = 0.19, adjusted P = 0.010] and 5-year OS [60.7% (95% CI: 47.0–74.4) versus 82.1% (95% CI: 68.0–96.2) HR 0.38 (95% CI: 0.14–1.01), P = 0.053, adjusted P = 0.033 (adjusted for p53 mutations (15)] compared to patients with the variant genotype (Figure 1A and B). These findings are in line with the data observed by Pardini (7) and Xing (8), and confirm an association between CC genotype and poor outcome in CRC.

Conversely, no survival differences by genotype were observed in the group of patients who received Cetuximab in combination with chemotherapy and CRT. The 5-year PFS was 73.2% (95% CI: 60.3–86.1) in patients homozygous for the C allele and 64.6% (95% CI: 47.7–81.5) in patients carrying the G allele [HR 1.38 (95% CI: 0.61–3.13) P = 0.439]. In the same genotype groups, the 5-year OS rates were 82.2% (95% CI: 71.0–93.4) and 73.1% (95% CI: 57.0–89.2) [HR 1.34 (95% CI: 0.52–3.48) P = 0.548], respectively (Figure 1C and D). When we explored the effect of the addition of Cetuximab to chemotherapy and CRT in the CC carriers we noticed an improved 5-year PFS [73.2% (95% CI: 60.3–86.1) versus 54.6% (95% CI: 40.5–68.7) p: 0.036], and 5-year OS [82.2% (95% CI: 71.0–93.4) and 73.1% (95% CI: 57.0–89.2)] [HR 3.13) (95% CI: 1.30–7.48) P = 0.003] (Figure 2A and B).

| Table 1. miR-608 genotype in the entire study population and by treatment arm |
| --- |
| miR-608 genotype | CAPOX (n = 78) | CAPOX-C (n = 77) | All patients (n = 155) |
| --- |
| CC | 50 (64.1) | 45 (58.4) | 95 (61.3) |
| CG | 25 (32.1) | 30 (39.0) | 55 (35.5) |
| GG | 3 (3.8) | 2 (2.6) | 5 (3.2) |

CAPOX, Capecitabine + Oxaliplatin; CAPOX-C, Capecitabine + Oxaliplatin + Cetuximab.

| Table 2. Baseline patient characteristics by miR-608 genotype and treatment arm |
| --- |
| miR-608 genotype | CAPOX (n = 78) | CAPOX-C (n = 77) |
| --- |
| Gender |
| Male | 27 (54.0) | 17 (60.7) |
| Female | 23 (46.0) | 11 (39.3) |
| Age (years) |
| Median (range) | 66 (28–79) | 64 (35–75) |
| WHO PS |
| 0 | 23 (46.0) | 14 (50.0) |
| ≥1 | 27 (54.0) | 14 (50.0) |
| MRI high-risk features |
| T3c–T3d (≥5 mm) | 36 (72.0) | 18 (64.3) |
| T4 | 11 (22.0) | 8 (28.6) |
| CRM+/at risk | 28 (56.0) | 17 (60.7) |
| EMVI positive | 37 (74.0) | 21 (75.0) |
| Low lying tumor | 34 (68.0) | 20 (71.4) |
| Tumor mutations |
| KRAS | 21 (42.0) | 11 (39.3) |
| NRAS | 2 (4.0) | 2 (7.1) |
| KRAS/NRAS | 23 (46.0) | 13 (46.4) |
| BRAF | 0 | 0 |
| PI3KCA | 6 (12.0) | 1 (3.6) |
| TP53 | 24 (48.0) | 11 (39.3) |

CAPOX, Capecitabine + Oxaliplatin; CAPOX-C, Capecitabine + Oxaliplatin + Cetuximab; PS, Performance Status; WHO, World Health Organization.
In the CG/GG carriers, who appear to have a better prognosis compared to CC carriers, addition of Cetuximab to chemotherapy and CRT was not associated with any clinical benefit: 5-year PFS in the CAPOX arm was 54.6% (95% CI: 40.5–68.7) versus 52.0% (95% CI: 47.7–67.1) in the CAPOX-C arm [HR 0.92 (95% CI: 0.45–1.90), P = 0.832]; 5-year OS were 62.3% (95% CI: 48.0–76.3) and 58.6% (95% CI: 44.2–72.9), respectively [HR 1.05 (95% CI: 0.43–2.59), P = 0.912] (Supplementary Figure 1, available at Carcinogenesis Online). In contrast, combining Cetuximab with chemotherapy and CRT appeared to have a detrimental effect in carriers of the variant alleles, who were found to have an increased risk of distant relapse [Distant Relapse rate for the CAPOX-C arm 33.6% (95% CI: 17.8–50.2) versus 11.1% in CAPOX (95% CI: 4.5–27.8); HR 2.93 (95% CI: 1.05–8.00), P = 0.036] which translated into worse Distant Relapse Free Survival [CAPOX arm 85.0% (95% CI: 71.5–98.5) versus CAPOX-C 64.6% (95% CI: 47.7–81.5), HR 2.73 (95% CI: 0.87–8.59) with a trend towards a significant difference, suggesting that Cetuximab may not be suitable for all patients with the CG/GG genotype.

Table 3. Complete response by miR-608 genotype and treatment arm

| miR-608 genotype | CAPOX (n = 78) | CAPOX-C (n = 77) | P value (n = 155) |
|------------------|----------------|------------------|------------------|
| CC               | 6/50 (12.0)    | 8/45 (17.8)      | 0.56             |
| CG/GG            | 4/28 (14.5)    | 4/32 (12.5)      | 0.056            |

CAPOX, Capecitabine + Oxaliplatin; CAPOX-C, Capecitabine + Oxaliplatin + Cetuximab.
Our study is challenging.

Discussion

In this retrospective analysis of the EXPERT-C trial, we have shown that the miR-608 CC genotype is associated with worse outcome when compared to the CG/GG genotypes in rectal cancer patients treated with neoadjuvant systemic chemotherapy followed by CRT, surgery, and adjuvant chemotherapy. Interestingly, the addition of Cetuximab to this intensified treatment strategy seemed to rescue the poor prognosis of patients with the CC genotype. To our knowledge, our study is the first to evaluate the prognostic role of rs4919510 in a homogeneous cohort of rectal cancer patients. Our patient population was represented by a prospectively collected series of LARC patients with an extensive molecular characterisation.

Previous case-control studies have explored miR-608 in relation to survival in CRC patients, with conflicting results. Pardini et al. (7) studied a European cohort of CRC patients and confirmed the presence of an association between rs4919510 and survival only in patients with stage III CRC who received 5-Fluorouracil (5-FU) based adjuvant chemotherapy. They observed that carriers of the G allele were at significantly decreased risk of recurrence when compared with CC genotype carriers. Similarly, Xing et al. (8) found that this SNP was associated with favourable outcome in 319 patients who received FOLFOX adjuvant chemotherapy, while this association was not evident in 89 patients who did not receive chemotherapy.

Contrary to these studies, Lin et al. (9) reported that the rs4919510 SNP was associated with poor outcome in stage III CRC receiving 5-FU based adjuvant chemotherapy. Several factors may account for the discrepancy between Lin’s findings and our observations: (i) both the training and the replication set in Lin’s study included 10% African-American (and 10% other ethnicities) in whom GG genotype was shown to be more frequent; (ii) while our cohort included only rectal cancer, Lin and colleagues studied a mixed population of proximal and distal colon and rectal cancers with the latter being less than 50%. Despite their analysis included adjustment for tumor site, the proportion of rectal cancer in the 179 stage III CRC patients is not specified.

As Ryan (4) and colleagues pointed out, ethnicity may account for important discrepancies in the prediction of CRC risk and prognosis among different populations: they found a significant association between the variant GG genotype and increased risk of death in Caucasian patients. Conversely, in African-American patients, there was a trend for the GG genotype to be associated with decreased risk of death, although this did not reach statistical significance (HR GG versus CC 0.38, 95% CI, 0.13–1.13, P = 0.082, adjusted HR GG versus CC 0.36, 95% CI 0.12–1.07, P = 0.66). Unfortunately the study did not include clinical information related to treatment, making comparison with our study challenging.

MicroRNA expression is tissue and organ specific and differences in microRNA deregulation have been observed in rectal compared to colon cancers (18–21). It is likely that the effects of rs4919510 on the interaction between miR-608 and its wide spectrum of target mRNAs may also differ according to the location of the primary tumor, thus possibly explaining some divergent results observed among studies which included heterogeneous patient populations. Notably, as suggested by Ryan and colleagues, two genes involved in the fluoropyrimidine metabolism [thymidine kinase and folylpolyglutamate synthase (10,22)] have been included in the list of putative targets of miR-608 and may potentially account for the effects of rs4919510 on the modulation of response to fluorouracil when given in combination with other chemotherapy drugs or as a radio-sensitizer with radiotherapy.

All patients included in our study received the same treatment with the exception of the addition of Cetuximab for those randomised to the investigational arm. This allowed us to explore for the first time the potential association between rs4919510 and activity of this anti-epidermal growth factor (EGFR) monoclonal antibody. Notably, the administration of Cetuximab appeared to improve the outcome of patients with the CC genotype while no incremental benefit from its use was observed in the group of patients with the GG/GG genotypes. This resulted in a statistically significant interaction for survival between Cetuximab treatment and miR-608 genotype. These findings suggest that rs4919510 may potentially interfere with the mechanism of action of Cetuximab ultimately leading to an attenuation of its anti-tumor properties. In support of this theory, miR-608 has been reported to target the EGFR and other genes which were previously shown to mediate resistance to EGFR inhibition such as MET (23). Alteration of binding affinities of mir-608 to these targets may explain the absence of Cetuximab benefit in carriers of the G allele.

Even though our cohort has been prospectively collected and is homogeneous in terms of ethnicity and treatment, we acknowledge that our study may have some limitations: (i) the analysis of rs4919510 was not originally planned when the EXPERT-C study was designed and therefore it suffers from all the limitations inherent to retrospective biomarker analyses; (ii) Given the investigational nature of both treatment arms of the EXPERT-C trial, it is not known whether the study findings are applicable to a rectal cancer patient population treated with standard fluoropyrimidine-based CRT.

In conclusion, we believe that our findings are of interest and support the importance of small non-coding RNAs as potential determinants of tumor aggressiveness and/or response to treatment in LARC patients prompting further analysis in this setting. An extensive analysis of microRNA expression in patients from the same series is ongoing.

Supplementary material

Supplementary Figure 1 can be found at http://carcin.oxfordjournals.org/

Funding

The work was supported by Cancer Research UK (CEA18052), European Union FP7 (CIG 334261) and the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) at The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research (grant A62) to N.V.

Acknowledgements

We acknowledge support from the NIHR BRC at The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research and from the Peter Stebbings Memorial Charity. Conflict of Interest Statement: D.C. received research funding from: Roche, Amgen, Celgene, Sanofi, Merck Serono, Novartis, AstraZeneca, Bayer, Merrimack and MedImmune. C.P. has had advisory roles with Sanofi. J.T. has had advisory roles with Amgen, Roche, Sanofi-Aventis, and Merck. A.C. has had advisory roles with Merck-Serono and Roche. He has received research funding from
Roche and honoraria from Roche and Merck-Serono. I.C. has had advisory roles with Merck Serono, Roche, Sanofi Oncology, Bristol Myers Squibb, Eli-Lilly, Novartis, Gilead Science. He has received research funding from Merck-Serono, Novartis, Roche and Sanofi Oncology, and honoraria from Roche, Sanofi-Oncology, Eli-Lilly, Taiho. All other authors declare no conflict of interest.

References
1. Glimelius, B. et al. (2013) Rectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann. Oncol., 24, vi81–8.
2. Lovat, F. (2011) MicroRNAs in the pathogenesis of cancer. Semin. Oncol., 38, 724–733.
3. Cech, T.R. et al. (2014) The noncoding RNA revolution—trashing old rules to forge new ones. Cell, 157, 77–94.
4. Ryan, B.M. et al. (2010) Genetic variation in microRNA networks: the implications for cancer research. Nat. Rev. Cancer., 10, 389–402.
5. Wojcicka, A. et al. (2014) MicroRNA-related sequence variations in human cancers. Hum. Genet., 133, 463–469.
6. Okugawa, Y. et al. (2014) An update on microRNAs as colorectal cancer biomarkers: where are we and what’s next? Expert Rev. Mol. Diagn., 14, 999–1021.
7. Pardini, B. et al. (2015) Polymorphisms in microRNA genes as predictors of clinical outcomes in colorectal cancer patients. Carcinogenesis, 36, 82–86.
8. Xing, J. et al. (2012) Genetic polymorphisms in pre-microRNA genes as prognostic markers of colorectal cancer. Cancer Epidemiol. Biomarkers Prev., 21, 217–227.
9. Lin, M. et al. (2012) Genetic polymorphisms in MicroRNA-related genes as predictors of clinical outcomes in colorectal adenocarcinoma patients. Clin. Cancer Res., 18, 3982–3991.
10. Ryan, B.M. et al. (2012) rs4919510 in hsa-mir-608 is associated with outcome but not risk of colorectal cancer. PLoS One, 7, e36306.
11. Dewdney, A. et al. (2012) Multicenter randomized phase II clinical trial comparing neoadjuvant oxaliplatin, capcitabine, and preoperative radiotherapy with or without cetuximab followed by total mesorectal excision in patients with high-risk rectal cancer (EXPERT-C). J. Clin. Oncol., 30, 1620–1627.
12. Sha, D. et al. (2014) Association study of the let-7 miRNA-complementary site variant in the 3’ untranslated region of the KRAS gene in stage III colon cancer (NCCTG N0147 Clinical Trial). Clin. Cancer Res., 20, 3319–3327.
13. Scalfani, F. et al. (2015) Prognostic role of the LCS6 KRAS variant in locally advanced rectal cancer: results of the EXPERT-C trial. Ann. Oncol., 26, 1936–1941.
14. Scalfani, F. et al. (2014) FcgammaRIIa and FcgammaRIIa polymorphisms and cetuximab benefit in the microscopic disease. Clin. Cancer Res., 20, 4511–4519.
15. Scalfani, F. et al. (2014) TP53 mutational status and cetuximab benefit in rectal cancer: 5-year results of the EXPERT-C trial. J Natl Cancer Inst., 106.
16. Scalfani, F. et al. (2014) RAS mutations and cetuximab in locally advanced rectal cancer: results of the EXPERT-C trial. Eur. J. Cancer, 50, 1430–1436.
17. van Huis-Tanja, L. et al. (2013) Concordance of genotype for polymorphisms in DNA isolated from peripheral blood and colorectal cancer tumor samples. Pharmacogenomics, 14, 2005–2012.
18. Slattery, M.L. (2011) MicroRNAs and colon and rectal cancer: differential expression by tumor location and subtype. Genes Chromosomes Cancer, 50, 196–206.
19. Gaedcke, J. et al. (2012) The rectal cancer microRNAome—microRNA expression in rectal cancer and matched normal mucosa. Clin. Cancer Res., 18, 4919–4930.
20. Koga, Y. et al. (2010) MicroRNA expression profiling of exfoliated colonicocytes isolated from feces for colorectal cancer screening. Cancer Prev. Res. (Phila), 3, 1435–1442.
21. Croce, C.M. (2009) Causes and consequences of microRNA dysregulation in cancer. Nat. Rev. Genet., 10, 704–714.
22. Agarwal, V. et al. (2015) Predicting effective microRNA target sites in mammalian mRNAs. eLife, 4:e05005
23. Zhang, Y. et al. (2014) MicroRNA-608 and microRNA-34a regulate chor- doma malignancy by targeting EGFR, Bcl-xL and MET. PLoS One, 9, e91546.