hsa-mir31-3p expression as predictor of Anti-EGFR response in KRAS wild type patients with metastatic colorectal cancer

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Abstract

In colorectal cancer, KRAS mutations are associated with resistance to anti-EGFR antibodies. A major challenge is to identify, in wild-type KRAS patients, markers that predicts response to this therapy. We focused on miRNAs, which can play a role in the resistance to anti-EGFR based chemotherapy for metastatic colorectal cancer (mCRC). We first analyzed 1145 miRNAs in 84 colorectal tumors and 5 normal colon mucosae. We then conducted a study with 3 subgroups of patients. Group 1 is a retrospective series of patients treated by cetuximab and irinotecan, group 2 is a prospective collection of patient treated by cetuximab or panitumumab based chemotherapy and group 3 is a series of patients prospectively treated by panitumumab and irinotecan as third-line. Using a Cox proportional hazards model, fitted using principal components analysis of group 1, we identified a predictive signature of 11 miRNA linked to disease free survival (p<0.01). Validation by RT qPCR showed that all the survival information is associated with miRNA hsa-mir-31-3p. We tested expression of this miRNA and the presence or absence of mutation BRAF on group 1 of 33 patients, in a Cox model, and found a hazard ratio (HR) of 1.9 CI95% [1.1-2.9]. In the two prospective series (38 patients) the prognostic impact of hsa-mir31-3p the HR is estimated to 1.9 CI95% [1.1-3.1]. We applied the multivariate model obtained from group 1 to group 2 and 3 in order to predict the disease free survival of the patients. The accuracy of the prediction measured by the AUC is 0.77. The performance of the test remains stable from 10 weeks to 48 weeks. A Cox proportional hazards model based on the hsa-mir31-3p logged expression, fitted using principal components from group 1 and BRAF mutation status as a clinical covariate allowed us to classify group 2 and 3 according to a free progression survival risk score (P=0.005) with a specificity of 62% [95% CI: 38%-82%] and a sensitivity of 82% [95% CI: 56%-96%] for the prediction model. We established a nomogram, taking into account mir-31-3p expression level, age, gender and BRAF mutation status, which predict the progression risk (P<0.0001). It is the first tool for selecting individual patients with a wild-type KRAS tumor for anti-EGFR therapy.

Patients

• The training set (group 1) was set up from a retrospective series of 33 patients, 24 males and 9 females with a KRAS wild-type tumors. The mean of age is 58.6 ±11.6 years. All these patients were refractory to irinotecan or oxaliplatin Among them, 24 received an association of cetuximab + irinotecan, 5 an association of cetuximab + 5FU + irinotecan, 2 an association of cetuximab + 5FU + oxaliplatine, 1 an association of cetuximab + xeloda and 1 an association of panitumumab + Irinotecan, 21 and 12 patients were classified as non responders and responders respectively according to RECIST criteria

•The validation sets were set up from two independent groups of 19 patients each prospectively collected with a KRAS wild type tumors:

- ➢Group 2, includes 19 patients refractory to irinotecan based chemotherapy, 11 males, 8 females, with a mean age of 67.6 ±11.6 years. Among them, 11 received an association of cetuximab + irinotecan, 4 an association of cetuximab + 5FU + irinotecan, and 4 a panitumumab monotherapy. 4 and 15 were classified as responders and non responders respectively.
- ➢ Group 3, includes 19 patients, 12 males, 7 females, with a mean age of 61.8 ±12.2 years. All received a panitumumab + irinotecan treatment in a framework of a phase II trial (PIMABI). 8 and 11 classified as responders and non responders respectively.

Method

✓We performed a global miRNA expression profiling of non-mutated KRAS colorectal tumor tissue samples using the Illumina Human microRNA Expression Profiling Assay v2 which measures the expression levels of 1145 miRNAs.

✓Small RNAs were extracted from frozen tumors using the mirVana miRNA Isolation Kit from Ambion. Global microRNA (miRNA) profiling was performed by labeling and hybridizing 750ng of extracted RNA from each sample on Illumina Human v2 microRNA.

✓Specific quantification of expression level of miRNA hsa-miR-31-3p was performed using specific TaqMan pre-designed assays on retrotranscribed RNA and a ABI7900HT Real-Time PCR System. Expression levels were normalized to the references nRNA RNU6B levels through the ΔΔCt method.

Survival model prediction

microRNA expression-based predictor of survival risk group was calculated by combining a Cox proportional hazards model and a supervised principal component method.

Univariate Cox proportional hazards model was used to categorize miRNAs for which log expression level is correlated with survival time.

Results

•Global miRNA expression profiling:

On the 1145 analysed miRNA, 11 show a correlation between its expression level and the prognosis.

• Real-time miRNA quantitative PCR analysis using specific TaqMan pre-designed assays was performed to quantify the expression levels. A fair correlation was noted between the hsa-miR-31-3p expression level measured by the two technologies with r= 0.88. Furthermore among all microRNA tested by qPCR only one (hsa-miR31-3p) on the training set of 33 patients exhibited significant different expression levels between tumor from bad prognosis and a good prognosis patients (fig.1)

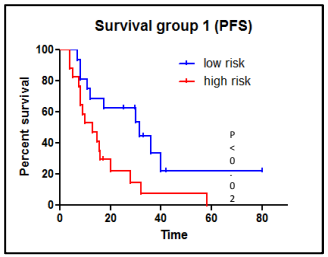
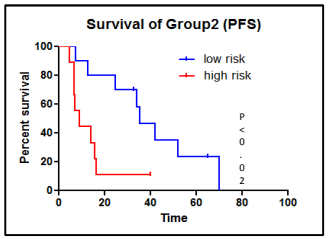


Fig.1: Kaplan-Meier model of progression free survival for 33 non-mutated KRAS patients with colorectal cancer, grouped by high expression or low expression of hsa-miR-31-3p.

• This micro RNA was tested on group 2 and group 3 and a significant correlation with survival was observed in each group (Fig. 2 and 3) and in the combined group 2 & 3 (Fig. 4).



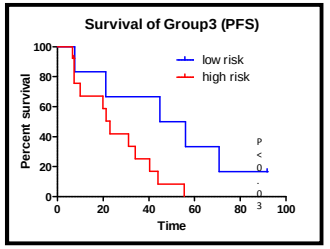


Fig.2 and 3: Kaplan-Meier model of progression free survival for 19 non-mutated KRAS patients with colorectal cancer in each group.

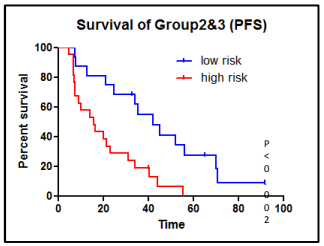


Fig.4: Kaplan-Meier model of progression free survival for 38 non-mutated KRAS patients with colorectal cancer, grouped by high expression or low expression of hsa- miR-31-3p.

• We used the training set (group 1) to develop a prediction model. The prediction accuracy of the model was quantified on the independent validation dataset (group 2 & 3) using:

i) the concordance index, which is numerically equivalent to the area under the receiver operating characteristic curve (0.7535888) and the performance of the test remains stable from 10 weeks to 48 weeks (Fig.5)

ii) a Univariate Cox Survival analysis done based on risk of progression score (p<0.0001)

• Finally multivariate Cox proportional hazards models with BRAF mutational status and miR31-3p expression as covariates (Fig 6) were used to construct a nomogram for PFS to predict the likelihood of progression (Fig. 7)

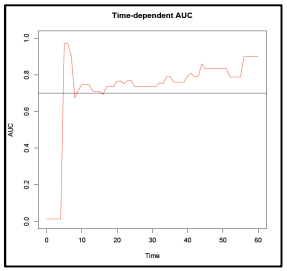


Fig.5: Value to the AUC according to the different PFS values tested.

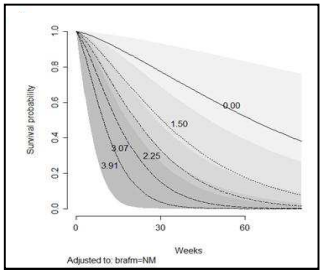


Fig.6: Multivariate Cox proportional hazards models with BRAF mutational status and has-miR31-3p expression as covariates.

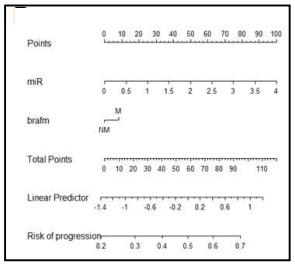


Fig.7: Nomogram for PFS constructed based on BRAF mutational status (brafm, NM: non mutated; M: mutated) and log. miR expression (miR). For each patient, points are allocated to each of the variable by selecting the corresponding points from the points scale, e.g. a patient with a mutated BRAF would score 8 points; a patient with log.miR = 0.5 would score 12 points. A sum of the points is then plotted on the Total Points scale which corresponds to a predicted rate of progression.

Conclusion

We showed the prognostic role of the expression of hsa-mir-31-3p in the response to anti-EGFR therapy in KRAS wild type metastatic colorectal cancer patients.

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