Data Article

Data on the evaluation of FGF2 gene expression in Colorectal Cancer

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ABSTRACT

The data presented in this article is related with the research paper entitled “Evaluation of MGP gene expression in colorectal cancer”, available on Gene journal [1]. From all the transcription factors known to regulate MGP, FGF2 is the most described in colon adenocarcinoma and colon tumor cell lines, where it was shown to: i) contribute for the invasiveness potential; and ii) promote proliferation and survival of colorectal cancer cells. These in vitro studies pose the hypothesis that FGF2 associated signaling pathways could be...
promoting the regulation of others genes, such as MGP, that may lead to tumor progression which ultimately could result in poor prognosis in colon adenocarcinoma.

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Specifications Table

| Subject | Molecular biology |
|---------|------------------|
| Specific subject area | Colorectal cancer, Molecular biology |
| Type of data | Table |
| | Graph |
| | Figure |
| How data were acquired | qRT-PCR, SPSS |
| Data format | Raw |
| | Analysed |
| Parameters for data collection | FGF2 was shown to be both a regulator of MGP and an inhibitor of cellular differentiation in colorectal cancer organoids and FGF family proteins were proven to have an important role on the survival and growth of stem cells during embryogenesis, carcinogenesis and tissue regeneration |
| Description of data collection | FGF2 gene expression analysis through qRT-PCR and assessment of the correlation with MGP gene expression and clinical and histopathological data analysis using SPSS software in colorectal patients |
| Data source location | University of Algarve |
| | Faro |
| | Portugal |
| Data accessibility | Data is available with this publication |
| Related research article | Caiado, H. et al. 2019 |
| | Evaluation of MGP gene expression in colorectal cancer |
| | Gene |
| | doi.org/10.1016/j.gene.2019.144120 |

Value of the Data

- The data presented here were obtained in order to evaluate FGF2 gene expression in patients with colorectal cancer. This data may be of great relevance in trying to understand how MGP gene expression deregulation may affect patients prognosis.
- Beneficiaries of these data are all those who seek knowledge about the molecular mechanisms that could be underlying MGP deregulation in tumorigenesis.
- These data report the upregulation of FGF2 gene expression in tumor tissue and its positive correlation with MGP gene expression in CRC. These results could provide future insights for the search of new therapeutic targets associated with MGP gene expression and its deregulation in cancer.

1. Data Description

The fibroblast growth factor (FGF) signaling network has been implicated in several pathways, such as normal cell growth, differentiation, angiogenesis and tumor development [2]. The transcription factor FGF2 is one of the most studied in terms of its role in carcinogenesis including its role in tumor cell differentiation and proliferation [2]. Moreover, it is known that FGF2 induces transcription of the MGP gene [3].

In this report, we describe data regarding the expression analysis performed by qRT-PCR for FGF2, for both normal and tumor tissues, of 23 out of 33 CRC patients [1] whose samples were
still available, and 9 samples from the control group (Fig. 1). The data showed that the expression of FGF2 was significantly up-regulated in CRC tissues compared to matched normal tissues (p=0.002). Our data is in accordance with what was already described in the literature regarding the increase of FGF2 expression in various tumor tissues, such as lung [4], colorectal [5], bladder [6] and prostate [7].

To evaluate if there is a correlation between FGF2 expression and the clinical-pathological features of the patients, we analyzed all the variables shown in Tables 1 and 2. No statistically significant associations were found between FGF2 expression and the clinical and pathological features of the patients.

We then evaluated the correlation between FGF2 and MGP expression. FGF2 mRNA expression determined by qRT-PCR was well correlated (r=0.572, p=0.004) with that determined for MGP [1] (Fig. 2).

In our previously published study, we found that the two step cluster analysis of the CRC samples allowed differentiating patients with a better or worse survival outcome [1]. Subsequently, we performed a multivariate classification of two step clusters [8] to determine possible patient profiles, taking into account the characteristics of categorical and numerical variables (Table 3). This type of analysis allows the exploitation of data taking into account each variable independently from each other’s, to try to identify homogeneous groups depending on their characteristics. Since we did not find any correlation between the high expression of FGF2 and the overall patient survival rate (Fig. 3), we then evaluated the prognostic value of different variables to differentiate patients in different groups according to the influence of these factors. The variables considered were: T classification, N classification, tumor staging, gender, deceased, fold change MGP categorized, fold change FGF2 categorized, fold change MGP, fold change FGF2, tumor histology, KRAS mutations, tumor location, survival rate (months), polyposis and stroke. According to this analysis, patients were divided into clusters 1 and 2. Patients in cluster 1 presented a stage N0 of lymph node metastasis (50%), the tumor was either in stage II (33.3%) or stage III (44.4%), mostly male (72.2%), with low MGP (72.2%) and FGF2 (55.6%) levels of expression, with a fold change for MGP of 3.09 (±3.03) and for FGF2 of 4.89 (±6.81), with a tumor histology showing either a moderately (44.4%) or well differentiated tumor (44.4%), without mutation on KRAS (61.1%), with a T3 classification (72.2%), with a mean survival time of 49.61 (±18.6) months, with the tumor mostly located in rectum (38.8%) and without the presence of polyposis (88.9) and no stroke (88.9%). Patients in cluster 2 presented a stage N1 of lymph node
Fig. 1. Relative $MGP$ and $FGF2$ gene expression in samples from patients with colon adenocarcinoma. Relative $MGP$ (A) and $FGF2$ (B) gene expression levels were analyzed by qRT-PCR in a total of 9 samples from control group and 23 samples from colorectal cancer tissue (normal and tumor mucosa). The latter showed higher mRNA levels of $MGP$ and $FGF2$ than non-tumor tissues ($MGP$ $p=0.002$; $FGF2$ $p \leq 0.001$). Values are presented as mean ± SD. The Mann-Whitney and Kruskal Wallis non parametric tests were performed for the statistical analysis.
Table 2
Histopathological features of patients

| Characteristics                  | MGP (n=23) |        | FGF2 (n=23) |        |
|----------------------------------|------------|--------|-------------|--------|
|                                  | Number (%) | Mean value | p value | Number (%) | Mean value | p value |
| Tumor Location                   |            |          |            |            |
| Rectum                           | 12 (52)    | 4.672   | 0.618      | 12 (52)    | 3.967     | 0.493   |
| Rectosigmoid Junction             | 3 (13)     | 6.217   |            | 3 (13)     | 2.479     |        |
| Ascending Colon                  | 2 (9)      | 2.633   |            | 2 (9)      | 10.730    |        |
| Sigmoid                          | 1 (4)      | 8.004   |            | 1 (4)      | 3.653     |        |
| Cecum                            | 2 (9)      | 2.793   |            | 2 (9)      | 14.938    |        |
| Hepatic Angle                    | 3 (13)     |          |            | 3 (13)     |           |        |
| Tumor Histology                  |            | 0.196   |            | 0.655     |           |        |
| Well Differentiated              | 10 (44)    | 4.014   |            | 10 (44)    | 3.400     |        |
| Moderately Differentiated        | 9 (39)     | 2.164   |            | 9 (39)     | 7.867     |        |
| Poorly Differentiated            | 1 (4)      | 24.042  |            | 1 (4)      | 5.530     |        |
| Mucinous                         | 1 (4)      | 8.004   |            | 1 (4)      | 3.653     |        |
| Mucinous Well Differentiated     | 2 (9)      | 6.028   |            | 2 (9)      | 3.054     |        |
| T classification                 | 0.201      | 0.815   |            | 0.336     | 0.447     |        |
| pT2                              |            | 3.983   |            | 4.186     | 4.866     |        |
| pT3                              | 18 (78)    | 4.763   |            | 18 (78)    | 5.918     |        |
| pT4                              | 1 (4)      | 2.055   |            | 1 (4)      | 6.109     |        |
| N classification                 | 0.372      | 0.372   |            | 0.592     | 0.447     |        |
| N0                               | 9 (39)     | 3.155   |            | 9 (39)     | 3.017     |        |
| N1                               | 8 (35)     | 5.626   |            | 8 (35)     | 6.717     |        |
| N2                               | 6 (26)     | 5.053   |            | 6 (26)     | 6.536     |        |
| M classification                 | 0.227      | 0.227   |            | 0.745     | 0.447     |        |
| M0                               | 18 (78)    | 3.294   |            | 18 (78)    | 5.505     |        |
| M1                               | 5 (22)     | 8.884   |            | 5 (22)     | 4.201     |        |
| Hepatic Metastasis               | 0.227      |          |            | 0.745     | 0.447     |        |
| Yes                              | 5 (22)     | 8.884   |            | 5 (22)     | 4.201     |        |
| No                               | 18 (78)    | 3.294   |            | 18 (78)    | 5.505     |        |
| Pulmonary Metastasis             | 0.158      |          |            | 0.198     | 0.987     |        |
| Yes                              | 2 (9)      | 14.057  |            | 2 (9)      | 8.597     |        |
| No                               | 21 (91)    | 3.600   |            | 21 (91)    | 4.900     |        |
| KRAS mutations                   | 0.728      |          |            | 0.265     | 0.447     |        |
| Yes                              | 8 (35)     | 4.022   |            | 8 (35)     | 7.826     |        |
| No                               | 15 (65)    | 4.770   |            | 15 (65)    | 3.833     |        |

Mann-Whitney U test

Mann-Whitney U test

The tumor was either in stage III (20%) or stage IV (80%), mostly female (80%), with high MGP (100%) and FGF2 (80%) levels of expression, with a fold change for MGP of 9.61 (±8.4) and for FGF2 of 6.38 (±5.0), with a well differentiated tumor histology (40%), without mutation on KRAS (80%), with a T3 classification (100%), with a mean survival time of 18.00 (±8.2) months, with the tumor located in rectum (100%) and without the presence of polyposis (100%) and no stroke (100%).

Moreover, we performed a Kaplan-Meier survival analysis to assess if MGP and FGF2 could be in fact good prognostic factors in terms of overall survival rate for the two groups of patients found in the two-step cluster analysis. Patients in cluster 2, which presented a worst prognosis, had a higher mortality rate when compared with patients in cluster 1 (log-rank test p<0.001) (Fig. 4).

From the analysis it was perceived that patients in cluster 2 had a worst prognosis, in the way that all of these patients presented a small survival rate, and higher tumor stages when compared with patients in cluster 1. It’s also worthy of note, that the variables that significantly contributed to the division of the patients were the tumor staging, the presence of high level
### Table 3

Multivariate analysis of predictor factors

| Characteristics                  | Cluster 1 (n=18, %) | Cluster 2 (n=5, %) | p value |
|----------------------------------|---------------------|--------------------|---------|
| **N Classification**             |                     |                    |         |
| N0                               | 9 (50)              | 0 (0)              | p=0.126 |
| N1                               | 5 (27.8)            | 3 (60)             |         |
| N2                               | 4 (22.2)            | 2 (40)             |         |
| **Tumor Staging**                |                     |                    |         |
| Stage I                          | 3 (16.7)            | 0 (0)              | p=0.05  |
| Stage II                         | 6 (33.3)            | 0 (0)              |         |
| Stage III                        | 8 (44.4)            | 1 (20)             |         |
| Stage IV                         | 1 (5.6)             | 4 (80)             |         |
| **Gender**                       |                     |                    |         |
| Male                             | 13 (72.2)           | 1 (20)             | p=0.05  |
| Female                           | 5 (27.8)            | 4 (80)             |         |
| **Deceased**                     |                     |                    |         |
| No                               | 18 (100)            | 0 (0)              | p=0.05  |
| Yes                              | 0 (0)               | 5 (100)            |         |
| **Fold change MGP categorized**  |                     |                    |         |
| High MGP                         | 5 (27.8)            | 5 (100)            | p=0.05  |
| **Fold change FGF2 categorized** |                     |                    |         |
| High FGF2                        | 8 (44.4)            | 4 (80)             | p=0.159 |
| **Fold Change MGP, mean (SD²)**  |                     |                    |         |
| MGP vs FGF2                      | 3.09(±3.03)         | 9.61(±8.4)         | p=0.05  |
| p=0.128                          | 4.89(±6.81)         | 6.38(±5.00)        | p=0.403 |
| **Tumor Histology**              |                     |                    |         |
| Well differentiated              | 8 (44.4)            | 2 (40)             | p=0.246 |
| Moderately differentiated        | 8 (44.4)            | 1 (20)             |         |
| Poorly Differentiated            | 0 (0)               | 1 (20)             |         |
| Mucinous                         | 1 (5.6)             | 0 (0)              |         |
| Mucinous well differentiated      | 1 (5.6)             | 1 (20)             |         |
| **KRAS mutations**               |                     |                    |         |
| No                               | 11 (61.1)           | 4 (80)             | p=0.433 |
| **T classification**             |                     |                    |         |
| T1                               | 0 (0)               | 0 (0)              | p=0.412 |
| T2                               | 4 (22.2)            | 0 (0)              |         |
| T3                               | 13 (72.2)           | 5 (100)            |         |
| T4                               | 1 (5.6)             | 0 (0)              |         |
| **Survival Rate (Months), mean (SD²)** | 49.61(±18.6)       | 18.00(±8.2)        | p=0.05  |
| **Tumor Location**               |                     |                    |         |
| Rectum                           | 7 (38.8)            | 5 (100)            |         |
| Rectosigmoid junction            | 3 (16.7)            | 0 (0)              |         |
| Ascending colon                  | 2 (11.1)            | 0 (0)              |         |
| Sigmoid                          | 1 (5.6)             | 0 (0)              |         |
| Cecum                            | 2 (11.1)            | 0 (0)              |         |
| Hepatic angle                    | 3 (16.7)            | 0 (0)              |         |
| **Polyposis**                    |                     |                    |         |
| No                               | 16 (88.9)           | 5 (100)            | p=0.435 |
| Stroke                           |                       |                    |         |
| No                               | 16 (88.9)           | 5 (100)            | p=0.435 |

**Boldfaced values - Variables with p ≤ 0.05**

1. Chi Square test
2. Standard Deviation
3. Mann-Whitney test
4. Log Rank test
5. Spearman coefficient correlation test
Fig. 2. Correlation between FGF2 and MGP gene expression in tumor tissue. As described in experimental design in materials and methods, the correlation between MGP and FGF2 gene expression was evaluated through the SPSS software, applying the Spearman coefficient correlation test in the tumor tissue and establishing a positive and significant correlation between expression of both genes ($r=0.572; \ p=0.004$).

of MGP, gender and the survival rate. This means that, per se, the high levels of FGF2 alone are not sufficient for the clustering of patients, but in combination with other multiple variables can profile the patients into groups with a better or worst prognosis.

Despite the presence of some patients in cluster 1 presenting a T staging of 3 or even 4, this does not mean that these patients will actually have an associated worst prognosis. In fact, it was already shown in the literature that patients who presented a tumor stage III could have a better prognosis than those with a tumor stage II. For example, according to the American Joint Committee (AJCC) staging manual [9], when TNM staging is being evaluated, the clinicians have to take into account the tumor size (T), the number of lymph node metastasis, and the presence of metastasis. The stage is then categorized according to the combination of those three major factors, but the prognosis of the disease is reflected by its combination with other external variables that may also contribute to a worst and better prognosis. The conclusion from this analysis shows that it is the combination of the multiple variables analyzed, together with the high expression of FGF2 in tumor tissue that can differentiate patients in two groups associated to a better or worst prognosis.
Fig. 3. Overall survival curve of patients with overexpression of FGF2. Patients with high FGF2 gene expression appear to have a lower survival rate although this was not statistically significant (p=0.179). Small vertical lines indicate the censored cases referring to the number of patients that have not reached the terminal event during the data collection. p-value was calculated by log-rank test.

2. Experimental Design, Materials, and Methods

In this report we present briefly the materials and methods used to obtain the data here described. To see a more detailed material and methods, please refer to [1].

2.1. Clinical, demographic and pathological characteristics of patients

Tissue samples, as well as clinical and pathological information, were obtained as described in the research article “Evaluation of MGP Gene Expression in Colorectal Cancer”.

Clinical, demographic and histopathological information regarding patients is depicted in tables 1 and 2.

2.2. qRT-PCR

Total RNA was extracted from fresh biopsies stored in RNALater (CRC (n=23) including normal adjacent tissue and healthy colonic tissue (n=9)). After quality and quantity measurements, cDNA synthesis was performed using 1 μg of the extracted RNA treated with RQ1 DNase (1U per μg of RNA; Promega) and M-MLV reverse transcriptase (ThermoFisher Scientific) according to manufacturer’s instructions.
The expression of mRNA for FGF2 was analyzed by $2^{-\Delta\Delta Ct}$ method and normalized with the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as reference gene. Primer sequences for GAPDH and FGF2 were as follows: GAPDH: forward: 5’-TCAACCGATTGTGCTATTTGGGCG-3’ and reverse: 5’-CTCGCTCCTGGAAGATGGTGATGGG-3’; FGF2: forward: 5’-CAAAAACGGGGGCTTCTTCCTG-3’ and reverse: 5’-CCATCTTCTTCATAGCCAGGTAAAC-3’.

Data were presented as the relative quantity of target mRNA normalized with GAPDH and relative to the mean expression of the control group. Please refer to the research article “Evaluation of MGP Gene Expression in Colorectal Cancer” for the analyses of expression of mRNA for MGP [1].

2.3. Statistical analysis

Statistical analysis was performed using SPSS software program version 25. Values for gene expression are presented as mean and standard deviation (SD) and two-sided $P$ value less than 0.05 was defined as statistically significant. Fold changes presented correspond to the ratio of the values from tumor mucosa versus normal mucosa. Comparisons between group variables and gene expression were estimated using non parametric statistical tests: Mann–Whitney U and Kruskal–Wallis.
The cutoff value to distinguish the patients with low and high MGP and FGF2 levels were estimated taking into account the median value of the fold change for both MGP and FGF2.

A multivariate classification of two step clusters [8] was performed to determine possible patient profiles, taking into account the characteristics of categorical and numerical variables (Table 3). This allowed the formation of cluster 1 (n=18) and cluster 2 (n=5). Spearman coefficients were considered to analyze the correlation between MGP and FGF2 fold change values by the interest groups, namely, clusters and tissue samples. Overall survival probability for two groups of patients (clusters 1 and 2) was calculated using the Kaplan–Meier method; intergroup differences were determined using a log-rank test. Logistic regression analysis and $\chi^2$ analysis were used to evaluate the independent influence of factors on the final prognosis.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi: 10.1016/j.dib.2020.105765.

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