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# Altered expression of NFY-C and RORA in colorectal adenocarcinomas

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ABSTRACT

NFY-C, a subunit of the transcription factor NFY, binds to the promoters of several eukaryotic genes, including cell cycle-related genes. RORA is a steroid hormone receptor implicated in a range of important cellular processes. We evaluated the expression of NFY-C and RORA in colorectal adenocarcinomas and normal colonic tissue. NFY-C expression was elevated in adenocarcinomas. Moreover, NFY-C mRNA levels correlated with time to disease progression, while NFY-C protein expression was significantly higher in metastatic disease. RORA expression was downregulated in CRC adenocarcinomas compared to normal controls and correlated with time to disease progression. The role of NFY-C and RORA in CRC merits further investigation.

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## Introduction

Colorectal cancer (CRC) is the third most common type of cancer with over one million cases worldwide and the second leading cause of cancer-related death in the Western World with a disease-specific mortality of approximately 33% (Wolpin and Mayer, 2008). The identification of prognostic and predictive markers of CRC is of great importance for the management of patients.

NFY-C (Nuclear Factor Y, subunit C) is one of the three subunits of a heteromeric complex of which the transcription factor NFY is composed. NFY binds with high specificity and affinity to the CCAAT sequence motif in the promoters and enhancers of many eukaryotic genes and regulates their transcription (Mantovani et al., 1989). In higher eukaryotes, CCAAT boxes are found in the promoters of developmentally controlled and tissue-specific genes, housekeeping and inducible, cell-cycle related genes, gene families co-ordinately activated during peptide presentation in antigen presenting cells and genes involved in cholesterol metabolism (Roy and Lee, 1995; Mach et al., 1996; Ronchi et al., 1996; Marziali et al., 1997; Dooley, 1998; Mantovani, 1998). NFY-C mRNA levels are similar in different human tissues except in the testis, where the expression levels are higher (Bellorini et al., 1997).

The role of NFY in cancer has not been fully elucidated. It has been suggested that NFY plays an important role in the regulation of genes that are expressed in various types of cancer (Borghini et al., 2006; Boily et al., 2007). Moreover, the involvement of NFY in tumor metastasis and breast cancer progression has been noted (Niida et al., 2008; Thomassen et al., 2008). Furthermore, NFY appears to regulate the expression of cell-cycle and cell death related genes, suggesting its contribution to tumor cell proliferation (Lee and Pedersen, 2003; Ohno et al., 2011).

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RORA (Retinoic Acid Receptor-related Orphan Receptor A) is a member of the ROR steroid hormone receptor superfamily (Jetten, 2004). Four isoforms (RORA1–RORA4) can be derived from the RORA gene by the utilization of different promoters. These isoforms share common DNA and ligand binding domains, but differ in their N-terminal region and display a tissue specific distribution. RORA is involved in lipid metabolism, maintenance of circadian clock function and in immunomodulation, while it has a critical role in the development of the cerebellum (Giguere et al., 1994; Hamilton et al., 1996; Missbach et al., 1996; Boukhtouche et al., 2004; Sato et al., 2004). Little is known with regard to its role in cancer. Although RORA is expressed in normal breast, prostate and ovarian epithelium, it is frequently inactivated in tumors of these organs (Zhu et al., 2006; Gu et al., 2010). Moreover, in support of this differential expression, the RORA gene is located in the middle of the FRA15A, a common fragile site (CFS) located in 15q22.2 (Zhu

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**Table 1**
NFY-C and RORA mRNA expression in colorectal adenocarcinomas.

| Clinicopathological characteristics | | Number of patients (%) | Median NFY-C mRNA expression (range) | *p* value | Median RORA mRNA expression (range) | *p* value |
|---|---|---|---|---|---|---|
| Gender | Male | 49 (60.5%) | 4.42 (1.11–8.19) | 0.332 | 1.36 (0–3.56) | 0.792 |
| | Female | 32 (39.5%) | 4.23 (0.76–7.58) | | 1.11 (0–3.04) | |
| Age group | ≤65 | 42 (51.9%) | 4.67 (1.11–7.81) | 0.313 | 0.50 (0–4.96) | 0.174 |
| | >65 | 39 (48.1%) | 4.10 (0.76–8.19) | | 1.51 (0–4.34) | |
| Duke's stage | A | 1 (1.2%) | 7.01 | 0.586 | 2.68 | 0.698 |
| | B | 43 (53.1%) | 4.38 (0.76–8.19) | | 1.52 (0–3.47) | |
| | C | 36 (44.5%) | 4.23 (1.11–7.82) | | 0.50 (0–4.56) | |
| | D | 1 (1.2%) | 5.08 | | 0.00 | |
| Grade | I | 13 (16.0%) | 4.48 (1.58–6.88) | 0.714 | 2.03 (0–3.32) | 0.403 |
| | II | 63 (77.8%) | 4.24 (0.76–8.19) | | 0.56 (0–3.56) | |
| | III | 4 (6.2%) | 6.01 (1.78–7.29) | | 1.35 (0–1.76) | |
| Primary site | Right colon | 23 (27.8%) | 5.74 (2.63–8.19) | 0.05 | 0.00 (0–3.32) | 0.024 |
| | Left colon and sigmoid | 35 (44.4%) | 3.76 (0.76–8.01) | | 2.00 (0–3.56) | |
| | Rectum | 23 (27.8%) | 4.12 (1.11–7.58) | | 0.51 (0–2.68) | |
| Relapse | Yes | 31 (38.3%) | 4.86 (1.11–7.81) | 0.607 | 0.61 (0–3.56) | 0.916 |
| | No | 43 (53.1%) | 3.97 (0.76–8.19) | | 1.16 (0–3.47) | |

et al., 2006). CFSs are highly unstable and recombinogenic regions of the genome, thus playing a mechanistic role in the initiation or progression of human cancers (Buttel et al., 2004).

In our previous study, analysis of publicly available microarray data revealed elevated mRNA expression levels of NFY-C and RORA genes in stage B CRC patients who relapsed, compared to patients who did not relapse (Antonacopoulou et al., 2008). Based on this observation, we aimed to assess the expression profile of NFY-C and RORA in human CRC and to further elucidate their association with clinicopathological characteristics of the disease and patient prognosis.

## Materials and methods

### Tissue specimens

This study received ethical approval from the local Research Ethics Committee at the University Hospital of Patras, Greece. Ninety two neoplastic formalin-fixed paraffin-embedded (ffpe) colonic tissue specimens were obtained from 92 CRC patients, who had undergone curative resection in the University Hospital of Patras. From the same patients, 53 normal colonic ffpe samples were also available. The clinicopathological characteristics of the patients are shown in Tables 1 and 2

Post-operative chemotherapy consisted of a six-week course of leucovorin, 200 mg/m$^2$ as a 2 h intravenous infusion, and an intravenous bolus of 5-fluorouracil, 500 mg/m$^2$ weekly, followed by a two week rest, for four cycles. Patients with rectal cancer were treated afterwards with pelvic radiotherapy and 5-fluorouracil, at a dose of 400 mg/m$^2$ on the first three and the last three days of radiotherapy. Chemotherapy started again 2–4 weeks after completion of radiotherapy and 5 more cycles were administered, each of which consisted of a four-week course of leucovorin and 5-fluorouracil, followed by a two-week rest period.

### RNA preparation

RNA was extracted from the 81 neoplastic and 53 normal colonic ffpe tissue specimens with the classical method of phenol/chloroform/proteinase K. The extracted RNA was digested with DNase (Ambion) to remove DNA and then it was quantified using Ribogreen (Molecular Probes, Leiden, The Netherlands) and the MX3000p (Stratagene, La Jolla, CA, USA).

### cDNA synthesis

cDNA was synthesized from the extracted RNA (1600 ng RNA from each sample) using Superscript III reverse transcriptase

**Table 2**
NFY-C and RORA protein expression in colorectal adenocarcinomas.

| Clinicopathological characteristics | | Number of patients (%) | Mean NFY-C protein expression | *p* value | Mean RORA protein expression | *p* value |
|---|---|---|---|---|---|---|
| Gender | Male | 55 (59.8%) | 27.89 (0–51.2) | 0.796 | 25.69 (16.6–40.5) | 0.095 |
| | Female | 37 (40.2%) | 29.19 (8.4–49.1) | | 23.37 (10.2–45.3) | |
| Age group | ≤65 | 51 (55.4%) | 27.59 (0–51.2) | 0.414 | 25.22 (16.6–37.4) | 0.345 |
| | >65 | 41 (44.6%) | 29.44 (2.2–49.1) | | 24.17 (10.2–45.3) | |
| Duke's stage | A | 1 (1.1%) | 26.10 | 0.011 | 18.7 | 0.453 |
| | B | 40 (43.5%) | 28.39 (1.4–51.2) | | 24.34 (10.4–45.3) | |
| | C | 45 (48.9%) | 27.12 (0–48.3) | | 25.35 (10.2–40.5) | |
| | D | 6 (6.5%) | 38.68 (23.2–49.1) | | 24.32 (20.6–29.2) | |
| Grade | I | 13 (14.1%) | 28.45 (8.6–46.2) | 0.483 | 25.86 (20.7–31.2) | 0.433 |
| | II | 71 (77.1%) | 27.24 (0–48.3) | | 24.60 (10.2–45.3) | |
| | III | 5(5.4%) | 33.56 (19.4–49.1) | | 25.98 (17.7–36.2) | |
| Primary site | Right colon | 23 (25%) | 36.16 (2.2–51.2) | <0.001 | 23.98 (10.2–40.5) | 0.255 |
| | Left colon and sigmoid | 45 (48.9%) | 26.02 (0–48.3) | | 24.07 (10.4–37.4) | |
| | Rectum | 24 (26.1%) | 25.48 (19.4–49.2) | | 26.84 (17.7–45.3) | |
| Relapse | Yes | 31 (33.7%) | 28.44 (0–48.3) | 0.458 | 24.04 (12.5–35.7) | 0.415 |
| | No | 40 (43.5%) | 25.96 (2.2–51.2) | | 25.53 (10.2–45.3) | |

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