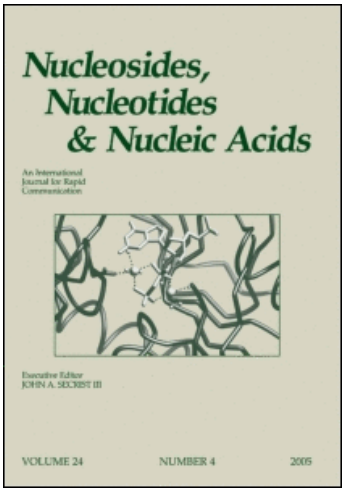

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Adenosine Kinase Gene Expression in Human Colorectal Cancer

S. Giglioni[a]; R. Leoncini[a]; E. Aceto[b]; A. Chessa[b]; S. Civitelli[b]; A. Bernini[b]; G. Tanzini[c]; F. Carraro[c]; A. Pucci[a]; D. Vannoni[a]
[a] Dipartimento di Medicina Interna, Sc. Endocrino-Metaboliche e Biochimica, Università di Siena, Italy [b] Dipartimento di Chirurgia, Università di Siena, Italy [c] Dipartimento di Fisiologia, Università di Siena, Italy

To cite this Article Giglioni, S. , Leoncini, R. , Aceto, E. , Chessa, A. , Civitelli, S. , Bernini, A. , Tanzini, G. , Carraro, F. , Pucci, A. and Vannoni, D.(2008) 'Adenosine Kinase Gene Expression in Human Colorectal Cancer', Nucleosides, Nucleotides and Nucleic Acids, 27: 6, 750 — 754
To link to this Article: DOI: 10.1080/15257770802145629
URL: http://dx.doi.org/10.1080/15257770802145629

Nucleosides, Nucleotides, and Nucleic Acids, 27:750–754, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802145629

Taylor & Francis
Taylor & Francis Group

ADENOSINE KINASE GENE EXPRESSION IN HUMAN COLORECTAL CANCER

S. Giglioni,[1] R. Leoncini,[1] E. Aceto,[2] A. Chessa,[2] S. Civitelli,[2] A. Bernini,[2] G. Tanzini,[3] F. Carraro,[3] A. Pucci,[1] and D. Vannoni[1]

[1]*Dipartimento di Medicina Interna, Sc. Endocrino-Metaboliche e Biochimica, Università di Siena, Italy*
[2]*Dipartimento di Chirurgia, Università di Siena, Italy*
[3]*Dipartimento di Fisiologia, Università di Siena, Italy*

□ *Real-time reverse transcription polymerase chain reaction (qRT-PCR) was used to evaluate gene expression of adenosine kinase, a key enzyme in adenosine metabolism, in human intestinal biopsy specimens of 10 colorectal cancer patients. Quantitative mRNA expression levels were normalized against the reference gene β-actin. The results showed that 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.*

Keywords Adenosine kinase; qRT-PCR; purine metabolism; colorectal cancer

INTRODUCTION

Colorectal cancer (CRC) is the second most common cause of cancer deaths worldwide, with 945,000 new cases and 492,000 CRC related deaths in 2000.[1,2] The incidence of CRC seems to be slightly higher in males, especially at ages between 50 and 70 years.[3] Resection of the primary cancer is the most common curative treatment for CRC,[4] but the management of patients with metastatic CRC has improved dramatically in the last decade, with increased chances of prolonged survival. In particular, postoperative combination chemotherapy regimens, including the use of adjuvant treatment with 5-fluorouracil plus leucovorin, have increased survival by >20% compared to no adjuvant treatment, and addition of either irinotecan or oxaliplatin,[5,6] has increased survival by another 10%. An additional increase in survival has resulted from use of the anti-angiogenic drug avastin (bevazucimab),[7,8] the EGFR-directed treatment with the antibody, cetuximab, holds additional promise in this regard.[9,10]

Address correspondence to Daniela Vannoni, Department of Internal Medicine, Endocrine-Metabolic Science and Biochemistry, Siena University, Siena, Italy. E-mail: vannoni@unisi.it

Although these therapeutic agents, alone or in combination, have led to advances in colorectal cancer management, most genetic factors and molecular mechanisms that, along with lifestyle-related risk factors, contribute to the development of adenomatous polyps and subsequent malignant transformation remain unknown. Therefore, studies in a variety of areas of investigation are warranted to find new strategies that will improve disease outcomes. This paper represents an approach to the study of purine metabolism in CRC, using real-time reverse-transcription polymerase chain reaction (qRT-PCR), a potent analytical tool for the detection of mRNA targets in tissues and body fluids.

We have evaluated expression of adenosine kinase (AdK; EC: 2.7.1.20) in human intestinal biopsy specimens. AdK is an enzyme contributing to steady-state maintenance of adenosine levels, which, in turn, have been implicated in the promotion of tumor cell proliferation.[11,12]

MATERIALS AND METHODS

Colorectal tissue samples were obtained from 10 patients (5 males, 5 females, aged 46–80 years, hospitalized in the Department of General Surgery of the University of Siena) during surgical resection for therapeutic purposes. Small biopsies were taken from non-necrotic proliferative regions, the tumor center and nontumor intestinal tissue less than 5 cm (close) and at least 10 cm (far) from the outer edge of the tumour. Biopsies were immediately placed in *RNA later* solution and stored at −20°C until studies. All biopsy specimens were analyzed at the Pathology Anatomy Department of the University of Siena, for the pathologist to confirm the TNM classification according UICC-1997 criteria. Microscopic analysis demonstrated that normal-close mucosa was not contaminated by tumor tissue.

Total cellular RNA was extracted from 25 mg tissue, using the classic TriZol-reagent protocol,[13]and the corresponding cDNA was synthesized starting from 1 μg RNA using the BioRad iSCRIPT cDNA Synthesis kit. Real-time amplification was prepared using iQ SYBR Green Supermix (Biorad).

β-actin (ACTB), which showed minimal variation between paired normal colon and cancer tissue, was chosen as a housekeeping reference gene and used to normalize mRNA levels among different samples.[14] The primers for amplification of AdK and β-actin genes were designed using the Primer 3 software[15] following standard criteria and were purchased from Sigma-Aldrich.

The experiments of gene expression were made in triplicate and values were expressed as relative quantification, normalized to the reference gene ACTB, ± SD. Normal-far mucosa was used as calibrator. Analysis of relative gene expression was carried out using the $2^{-\Delta\Delta Ct}$ method.[16]

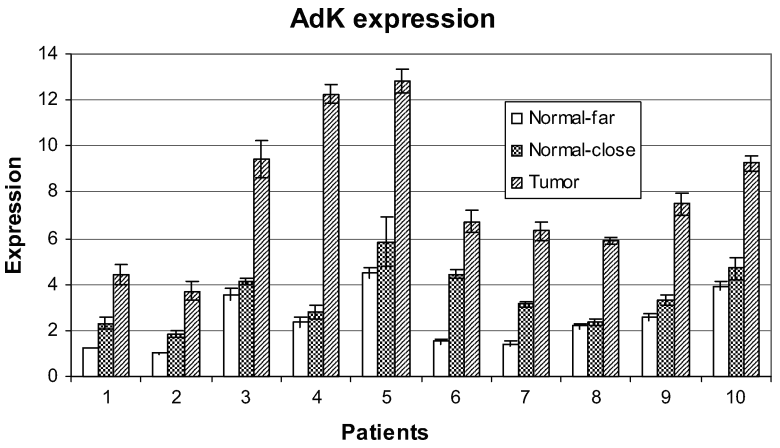

FIGURE 1 Distribution of AdK expression in tumor, normal-far and normal-close mucosa of 10 patients (numbered from 1 to 10) with colorectal cancer. The data were the means ± SD of three experiments. Values were normalized to the reference gene ACTB and relative gene expression was carried out using the $2^{-\Delta\Delta Ct}$ method, as reported in Materials and Methods section.

RESULTS

Figure 1 reports the distribution of the AdK expression in tumor, normal-far, and normal-close mucosa from each patient. AdK mRNA was significantly over-expressed in the tumour samples from all patients. Correlation of AdK gene expression with TNM classification was not significant in this small number of cases.

DISCUSSION

Tumor growth is a multifactorial process which clearly involves physiological and metabolic changes at the primary and metastatic sites of disease. Understanding the molecular alterations underlying changes in cancer cell metabolism is required in order to define new biomarkers and treatment targets. For example, gene expression array-based studies analyzing CRC tumors compared with corresponding noncancerous colonic epithelia[17] have identified clusters of genes showing differential expression in normal and tumor tissue.

Adenosine is a metabolite with multiple homeostatic and protective functions. High concentrations of adenosine have been demonstrated in solid tumors[11,12] but the mechanisms by which this occurs are not well understood. It is unclear whether adenosine is merely a passive product of necrosis and ischemia or whether it is released as the result of specific genetic alterations occurring during tumor progression. It has been suggested that adenosine may be a factor contributing to angiogenesis and tumor growth.[11,12]

Three enzymes maintain intracellular adenosine concentrations in steady-state: endo-5′-nucleotidase, adenosine deaminase and adenosine kinase and their coordinated changes may be required to generate adenosine. The literature provides some reports on the behaviour of purine metabolic enzymes in several types of malignancies.[18,19] Increased levels of adenosine in carcinoma or hypoxic cell lines have been attributed inhibition of adenosine kinase and the activation of 5′-nucleotidase.[12,20] However, our prior studies on cancerous intestinal mucosa, demonstrated that AdK enzymatic activity in vitro is significantly higher than in control mucosa,[21] and the current confirms increased adenosine kinase gene expression in colorectal tumor mucosa but not in surrounding unaffected mucosa. We believe that the role of this enzyme in the behaviour of colorectal tumors warrants further study.

REFERENCES

1. Parkin, D.M. Global cancer statistics in the year 2000. *Lancet Oncol.* **2001**, 2, 533–543.
2. Bae, J.M.; Park, J.G. Annual report of the Korea Central Cancer Registry Program 2000: based on registered data from 131 hospital. *Cancer Res. Treat.* **2002**, 34, 77–83.
3. Banerjea, A.; Clark, S.; Dorudi, S. The changing face of familial colorectal cancer. *Biol. Mol. J.* **2005**, 330, 2–3.
4. National Cancer Institute. What you need to know about cancer of the colon and rectum. Retrieved from http://www.cancer.gov/PDF/WYNTK/WYNTK_Colon.PDF
5. Saltz, L.B.; Cox, J.V.; Blande, C.; Rosen, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N. Engl. J. Med.* **2000**, 343, 905–914.
6. Goldberg, R.M.; Sargent, D.J.; Morton, R.F.; Fuchs, C.S.; et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. *J. Clin. Oncol.* **2004**, 22, 23–30.
7. Chen, H.X.; Mooney, M.; Boron, M.; Vena, D.; et al. Phase II multicenter trial of bevacizumab plus fluorouracil and leucovorin in patients with advanced refractory colorectal cancer: an NCI Treatment Referral Center Trial TRC-0301. *J. Clin. Oncol.* **2006**, 24, 3354–3360.
8. Kwon, H.-C.; Oh, S.Y.; Lee, S.; Kim, S.-H.; Kim, H.-J. Bevacizumab plus infusional 5-fluorouracil, leucovorin and irinotecan for advanced colorectal cancer that progressed after oxaliplatin and irinotecan chemotherapy: A pilot study. *World J. Gastroenterol.* **2007**, 13, 6231–6235.
9. Wong, S.F. Cetuximab: an epidermal growth factor receptor monoclonal antibody for the treatment of colorectal cancer. *Clin. Ther.* **2005**, 27, 684–694.
10. Cunningham, D.; Humblet, Y.; Siena, S.; Khayat, D.; et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* **2004**, 351, 337–345.
11. Spychala, J. Tumor promoting functions of adenosine. *Pharmacol. Therapeut.* **2000**, 87, 161–173.
12. Mujoomdar, M..; Hoskin, D.; Blay, J. Adenosine stimulation of the proliferation of colorectal carcinoma cell lines Roles of cell density and adenosine metabolism. *Biochem. Pharmacol.* **2003**, 66, 1737–1747.
13. Chomczynski, P.; Sacchi, N. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **1987**, 162, 156–159.
14. Rubie, C.; Kempf, K.; Hans, J.; Su, T.; Tilton, B.; et al. Housekeeping gene variability in normal and cancerous colorectal, pancreatic, oesophageal, gastric and hepatic tissue. *Mol. Cell. Probes* **2005**, 19, 101–109.
15. Primer 3. Retrieved from http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi
16. Schefe, J.H.; Lehmann, K.E.; Buschmann, I.R.; Unger, T.; Funke-Kaiser, H. Quantitative real-time RT-PCR data analysis: current concepts and novel "gene expression's Ct difference" formula. *J. Mol. Med.* **2006**, 84, 901–910.

17. Birkenkamp-Demtroder, K.; Christensen, L.L.; Harder Olesen, S.; Frederiksen, C.M.; et al. F. Gene Expression in Colorectal Cancer. *Cancer Res.* **2002**, 62, 4352–4363.
18. Eroglu, A.; Canbolat, O.; Demirci, S.; Kocaoglu, et al. Activities of adenosine deaminase and 5′-nucleotidase in cancerous and non cancerous human colorectal tissues. *Med. Oncol.* **2000**, 17, 319–324.
19. Natsumeda, Y.; Lui, M.S.; Emrani, J.; Faderan, M.A.; et al. Purine enzymology of human colon carcinomas. *Cancer Res.* **1985**, 45, 2556–2559.
20. Ulrich, K.; Decking, M.; Schlieper, G.; Kroll, K.; Schrader, J. Hypoxia-induced inhibition of adenosine kinase potentiates cardiac adenosine release *Circ. Res.* **1997**, 81, 154–164.
21. Vannoni, D.; Bernini, A.; Carlucci, F.; Civitelli, S.; et al. Enzyme activities controlling adenosine levels in normal and neoplastic tissues. *Med. Oncol.* **2004**, 21, 187–195.