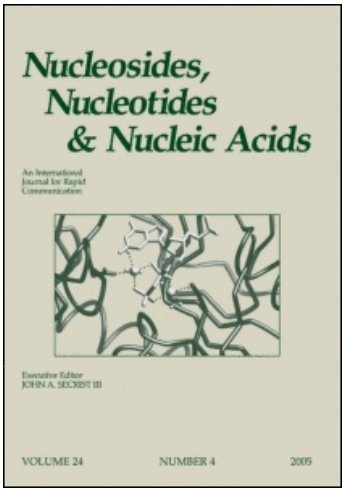

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 27 October 2004

To cite this Article Nicola, Maurizio Di , Santoleri, Fiorenzo , Soscia, Stefano , Fioroni, Massimiliano , Rubini, Corrado , Piattelli, Adriano and Spoto, Giuseppe(2004) 'Cyclic Guanosine Monophosphate Role in Human Carcinoma Pathogenesis', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1555 — 1558
To link to this Article: DOI: 10.1081/NCN-200027778
URL: http://dx.doi.org/10.1081/NCN-200027778

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1555–1558, 2004

Cyclic Guanosine Monophosphate Role in Human Carcinoma Pathogenesis

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ABSTRACT

In order to examine the cyclic nucleotides (cGMP) role in carcinoma growth and invasivity. We analyzed two cell lines, LSHT29 and 17GT, and tissues in patients with carcinoma and malignant tissues with (N^+) and without (N^-) lymph node metastases. Higher cGMP levels in pathological samples suggest a strong correlation between intracellular cGMP concentration and carcinoma progression.

Key Words: Cyclase; Phosphodiesterases; cGMP; cAMP; Cell carcinoma.

INTRODUCTION

Cyclic guanosine monophosphate is an essential second messenger for different cellular signals and is hydrolyzed to guanosine 5′-monophoshate by phosphodiesterases (PDEs), whose activity is regulated by different inputs originated from some

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DOI: 10.1081/NCN-200027778
1525-7770 (Print); 1532-2335 (Online)

signaling-systems. In this study, we look for a correlation between the cGMP and human cancer progression. Although cyclic nucleotide's role in carcinoma growth and invasivity has not been clearly defined, their function as second messengers of many molecular signals suggest that they may be key factors in neoplastic pathogenesis.

MATERIALS AND METHODS

cGMP concentration was measured with reverse-phase HLPC using the method of Spoto et al. with minor modification[1,2] and was confirmed by immune-enzymatic analysis.[3]

Samples

Twenty cases of human Oral Squamous Cell Carcinoma, OSCC, of the gingiva were used in the present study. Ten patients presented negative lymph nodes (N^-) while in ten patients the lymph nodes were positive (N^+). As control tissues, 18 specimens removed during third molar extractions were used.

The colon carcinoma cell lines (LS174T and HT29), were cultured as monolayers in a humidified incubator at 37°C in 5% CO_2—95% air and routinely grown in DMEM medium supplemented with 10% heat-inactivated fetal calf serum. All reagents were purchased from Seromed (Germany). The cells were split 1:3 twice a week. 80% confluence was reached at day 3 in T-75 tissue culture flask. Cells were count after trypsinization. Standard deviation never exceeded 5% and cell were always >95% viable, as shown by trypan-blue exclusion. Burker cell counts are expressed as the mean of triplicate counts. Cell cycle kinetics was determined by flow cytometry ''Epics Elite Coulter,'' with the aid of DNA fluorochromes. These methods are used to estimate the proportion of cells in the G1 cells, S and G2/M phases of the cell cycle from the DNA distribution.

Protein Content

Protein content was determined using a bicinchoninic acid protein determination kit from Sigma with bovine serum albumin as a standard.

Data Processing

Fischer's PLSD, Scheffe and Bonferroni–Dunn tests were used to evaluate the presence of statistically significant differences.

RESULTS

We analyzed the intracellular concentrations of cGMP, in the OSCC tissues. The mean value of $[cGMP]_i$ in 18 specimens retrieved from healthy patients was 0.35 μmol/l (±0.38 SD). For the healthy tissues, around the cancer, the cGMP concentration was 0.36 μmol/l (±0.70 SD). For the N^- group, the cyclic nucleotide concentration

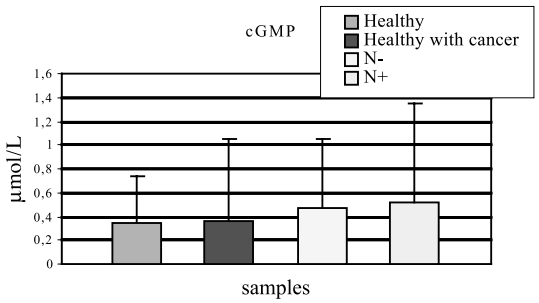

Figure 1. Cyclic guanosine monophosphate (cGMP) values in human carcinoma pathogenesis.

was 0.48 µmol/l (±0.57 SD). In the N^+ patients, the value of cGMP was 0.52 µmol/l (±0.83 SD) (Fig. 1). Data processing showed not statistically differences in cGMP concentration values.

We, also, analyzed two different cell lines, LSHT29 and 17GT. The average value of cGMP in LSHT29 cell lines was 0.1 µmol/l, while, the mean value of cGMP in 17GT was no detectable.

DISCUSSION

At first we analyzed two cell lines, characterized by different differentiation degrees. The values showed an elevated cGMP concentration in LSHT29 against 17GT which the cGMP concentration was no detectable.

Next we analysed Oral Squamous Cell Carcinoma samples and evaluated healthy control, healthy tissues in patients with carcinoma and malignant tissues with (N^+) and without (N^-) lymph node metastases. Examining these findings, we observed an increasing intracellular cGMP concentration with a higher cancer progression. Therefore, cGMP is not only an important second messenger of different cellular signals, generated by receptor coupled G-proteins, but could play an important role in growth, proliferation and cellular differentiation. Our data demonstrate as the higher cGMP levels in pathological samples suggest a strong correlation between intracellular cGMP concentration and carcinoma progression. Particularly, is important to notice that other studies showed that cyclic adenosine monophosphate (cAMP), another intracellular second messenger, was not present in OSCC. Therefore we support that this data may suggest a probably cGMP involvement in human carcinoma proliferation mechanism.

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