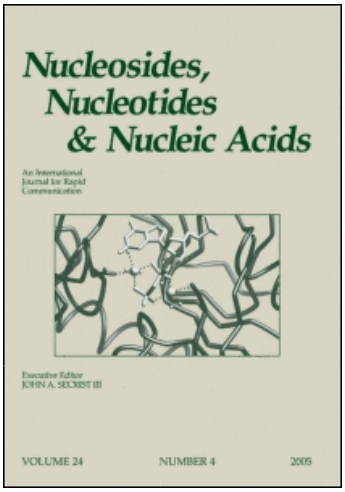

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Urinary Modified Nucleosides as Tumor Markers

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ABSTRACT

Extracts of urinary nucleosides have been sequentially purified and examined by mass spectrometric analysis. Seventeen modified nucleosides have been unequivocally identified and a further five provisionally identified. While several nucleosides were found only in a small number of extracts, the occurrence and levels of others were found to correlate with the tumour type and stage.

Key Words: Urinary nucleosides; Cancer; Modified nucleosides; Mass spectrometry.

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BACKGROUND

The levels of the modified nucleosides excreted in the urine have been shown to correlate with the progression of a number of different cancer types. Unlike the major nucleosides these modified nucleosides, originating primarily from transfer RNA, have no degradation or salvage pathways associated with them, and so are excreted as nucleosides in the urine.

EXTRACTION AND PURIFICATION OF URINARY NUCLEOSIDES

The optimized sequential protocol we have developed comprises a centrifugation, acidification and neutralization step, followed by application of a phenylboronate affinity chromatography column and finally further separation on an acidic cation exchange column and a basic anion exchanger,[1] providing a sequential, routine and reliable means of partial purification of urinary nucleosides producing a clean-up not available by a single HPLC separation or on-line LC/MS system.

MASS SPECTROMETRIC ANALYSIS

We have been developing and utilizing separation techniques linked to mass spectrometric detection in order to overcome the problems associated with past identification and quantitation methods. The three methods of analysis utilised were Gas Chromatography/Mass Spectrometry (GC/MS), High Performance Liquid Chromatography/Ion Trap Mass Spectrometry (HPLC/ITMS) and Capillary Liquid chromatography/Triple Quadruple Mass Spectrometry (CapLC/TQMS).[2,3]

NUCLEOSIDES IDENTIFIED IN URINE

17 nucleosides have been unequivocally identified in urine samples[3]:- 1-methyladenosine, cytidine, 5-methylcytidine, inosine, xanthosine, 1-methylinosine, N^1-ribosylpyrid-4-one-3-carboxamide, adenosine, guanosine, pseudouridine, dihydrouridine, N^2,N^2,7-trimethylguanosine, N^1-methylguanosine, N^2-methylguanosine, N^4-acetylcytidine, N^2,N^2-dimethylguanosine, and 5′-deoxy-5′-methylthioadenine. In addition 5 other modified nucleoside are provisionally identified as 5′-deoxycytidine, 5′-O-methylcytidine, methylacetylcytidine, ethylacetylcytidine, and 5′-O-formylcytidine.

VARIATION OF NUCLEOSIDE PROFILE WITH THE DISEASE STATE

Several nucleosides were found only to be present in only a small number of urine extracts. 5′-deoxycytidine was present only in a stage 4 head and neck patient,

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cytidine was found in ovarian stage 4, prostate stage 3, and head and neck stage 3 patients, 5-methylcytidine in head and neck and in ovarian stage 4 patients, ethylacetylcytidine was present in breast, bladder, ovarian, head and neck, lymphoma and prostatic patients, and formylcytidine and inosine were found in head and neck and ovarian patients. The other identified urinary nucleosides were found in all patient samples except for 1-methyladenosine, which was absent from 5 patient samples, 2 ovarian, and one head and neck, kidney and bladder patients.

VARIATION OF NUCLEOSIDE PROFILE WITH DISEASE PROGRESSION

Several showed changes in concentration that correlated with the stage of the disease. In each type of cancer, the levels of 1-methyladenosine, pseudouridine, 2-methylguanosine, N^2,N^2-dimethylguanosine, xanthosine, methylacetylcytidine and trimethylguanosine showed a progressive increase through stages 1, 2, 3 and 4, except in prostate cancer patients where 1-methyladenosine increased with stage to its highest at stage 3. The levels of acetylcytidine, adenosine and N^1-ribosyl-pyrid-4-one 3-carboxamide (PCNR) also increase through stages 1 and 2 to a maximum at stage 3 before falling at stage 4. In breast cancer the level of N^2,N^2-dimethylguanosine increased ten-fold in stages 3 and 4: in cancers of the prostate and breast the ratio of 1-methylguanosine to 2-methylguanosine increased with stage of the disease. In cancers except those of the breast, bladder and kidney, the ratio of 2-methylguanosine to xanthosine, of dimethylguanosine to xanthosine, and of trimethylguanosine to PCNR all increased with stage of the disease.

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