

AD-A112 398

OHIO STATE UNIV RESEARCH FOUNDATION COLUMBUS

F/0 6/1

CHEMICAL CARCINOGEN-INDUCED CHANGES IN TRNA METABOLISM IN HUMAN--ETC(U)

NOV 81 R W TREWYN

AFOSR-80-0283

NL

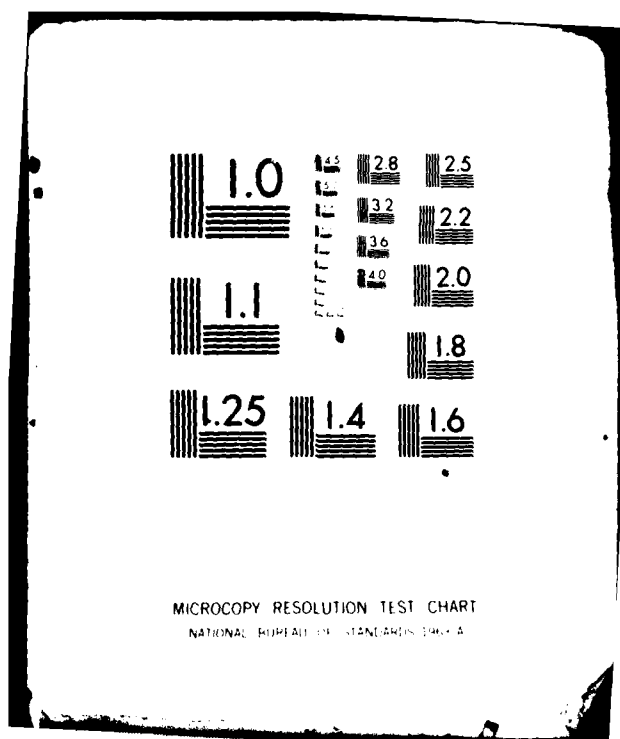
UNCLASSIFIED

AFOSR-TR-82-0156

[]
[]
[]



END
DATE
FILMED
4-82
DTIC



REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM	
1. REPORT NUMBER AFOSR-TR- 82 -0156	2. GOVT ACCESSION NO. AD-4112358	3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) CHEMICAL CARCINOGEN-INDUCED CHANGES IN tRNA METABOLISM IN HUMAN CELLS		5. TYPE OF REPORT & PERIOD COVERED Interim 10/1/80 to 9/30/81	
AUTHOR(s) Ronald W. Trewyn, Ph.D. Assistant Professor of Physiological Chemistry		6. PERFORMING ORG. REPORT NUMBER 762404/713292	
PERFORMING ORGANIZATION NAME AND ADDRESS The Ohio State University Research Foundation, 1314 Kinnear Road Columbus, Ohio 43212		8. CONTRACT OR GRANT NUMBER(s) AFOSR-80-0283	
CONTROLLING OFFICE NAME AND ADDRESS AIR FORCE OFFICE OF SCIENTIFIC RESEARCH /NL Building 410 Bolling Air Force Base, D.C. 20332		12. REPORT DATE November, 1981	
MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		13. NUMBER OF PAGES 3	
		15. SECURITY CLASS. (of this report) Unclassified	
		15a. DECLASSIFICATION DOWNGRADING SCHEDULE	
DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited.			
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) DTIC ELECTE MAR 24 1982			
18. SUPPLEMENTARY NOTES			
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Chemical carcinogens, tRNA metabolism, human cells, carcinogenesis, promotion of neoplasia, 1,2-dimethylhydrazine, 7-methylguanine, queuine.			
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Changes in nucleic acid catabolism were found in human cells transformed by an activated derivative of 1,2-dimethylhydrazine, with increased generation of 7-methylguanine being the major alteration. 7-Methylguanine was shown to inhibit the enzyme tRNA: guanine transglycosylase. This enzyme exchanges queuine (Q-base) for guanine in the first position of the anticodon of four different tRNA's, and tRNA's from transformed cells are Q-deficient. 7- Methylguanine was also found to induce Q-hypomodification of tRNA in normal			

DD FORM 1 JAN 73 1473

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ADA112358

FILE COPY

cells. It is felt that the changes discovered in nucleic acid catabolism and modification may be involved in the expression of carcinogenesis.

Accession For	
NTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By _____	
Distribution _____	
Availability Codes	
A 3	



Unclassified

RF Project 762404/713292

Report No.

INTERIM

REPORT

By

THE OHIO STATE UNIVERSITY
RESEARCH FOUNDATION

1314 KINNEAR RD.
COLUMBUS, OHIO 43212

To..... AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
Building 410
Bolling Air Force Base, D.C. 20332
Grant No. AFOSR-80-0283

On..... CHEMICAL CARCINOGEN-INDUCED CHANGES IN tRNA
METABOLISM IN HUMAN CELLS

For the period..... October 1, 1980 - September 30, 1981

Submitted by..... Ronald W. Trewyn
Department of Physiological Chemistry

Date..... November, 1981

Approved for public release;
distribution unlimited.

DEC 2 1981

AIR FORCE (1953)
NOTICE
This
appro
Dist.
MATTHEW J. KERPNER
Chief, Technical Information Division

b. Research Objectives

It was proposed that changes in tRNA metabolism are required for the progression of cells through the stages of carcinogenesis. Following the induction of the carcinogenic process, gradual phenotypic modulation towards a less differentiated cellular state occurs. Alterations in tRNA modification and catabolism commence soon after chemical carcinogen exposure, and the accruing changes in tRNA isoaccepting species may be involved in releasing the post-transcriptional controls over developmental gene expression. Our long-term objective is to elucidate the role of the altered tRNA metabolism in the dedifferentiation and progression of carcinogenesis.

c. Status of the Research

To examine changes in tRNA catabolism following carcinogen treatment of neonatal skin fibroblasts, appropriate methodology had to be developed. Cellular nucleic acids were radiolabeled for 24 hours with [³H]adenosine, [³H]guanine, or [³H-methyl]-methionine. The radiolabeling of proliferating cells was done in Serumless Medium to avoid purine catabolism by serum enzymes. Cell-derived catabolites in the medium were generally evaluated by high performance liquid chromatography (HPLC) at the end of the 24 hour period.

It was established early in the investigation that our improved HPLC method for the resolution and quantitation of modified nucleosides in the urine of cancer patients would not be particularly useful for the cell culture studies, since the free bases, rather than the nucleosides, are the predominant catabolites in vitro. A paried-ion reversed-phase HPLC procedure was developed which resolves most of the major and minor adenine catabolites radiolabeled in experiments with [³H]adenosine.

However, most of the results to date have been obtained with a newly developed HPLC gradient elution system for resolution of guanine and xanthine derivatives produced from [³H]guanine.

Comparison of nucleic acid catabolism by normal human fibroblasts and fibroblasts transformed with methylazoxymethyl alcohol (MAMA), an activated derivative of 1,2-dimethylhydrazine, was undertaken initially. Apparently the normal and transformed cells have functional hypoxanthine: guanine phosphoribosyl transferases, since both readily utilized [³H]-guanine for nucleic acid synthesis. Both cell types also generated xanthine as the predominant catabolite in long-term, pulse-chase experiments. However, the kinetics of catabolism differed, in that only the MAMA-transformed cells had generated appreciable [³H]xanthine after 24 hours.

Quantitative and qualitative differences in modified nucleic acid catabolites were also observed when proliferating normal and transformed human fibroblasts were compared. The nucleoside 7-methylguanosine was the predominant modified product secreted by the normal cells, while the free base 7-methylguanine was secreted preferentially by the MAMA-transformed cells. The transformed cells also produced small amounts of the nucleosides 7-methylguanosine and 1-methylguanosine.

The analyses of RNA catabolism by the cells in culture may be complicated further by the fact that the cells appear to remove 7-methylguanine from the culture medium during certain stages in cell cycle. Our preliminary results indicate that this uptake occurs prior to the DNA synthesis (S) phase of the cycle. The current understanding of the biochemistry of the modified guanines does not offer an explanation for this phenomenon, i.e., these "waste" products should be of no use to the cells. However, based on our previously demonstrated role for exogenous

1-methylguanine and 7-methylguanine in neoplastic transformation, this finding will have to be explored further.

Experiments were also initiated into the changes in tRNA modification associated with carcinogenesis. tRNA: guanine transglycosylase to be utilized to assay for queuine (Q)-deficient tRNA in transformed cells has been purified from an RNase-deficient strain of *E. coli*. Standardization of the method has been accomplished with Q-deficient tRNA's from yeast and cultured human trophoblastic tumor cells. Although the initial attempts to induce Q-hypomodification of tRNA in human fibroblasts were unsuccessful, it was discovered that the hamster cells transformed previously with methylated purines contained highly Q-deficient tRNA. In addition, we were able to generate Q-hypomodification of tRNA in normal hamster cells by treating the cultured cells with 7-methylguanine under conditions whereby 7-methylguanine will induce cell transformation. tRNA: guanine transglycosylase, the enzyme catalyzing the Q macromolecular tRNA modification, was also isolated from rabbit erythrocytes, and shown to be inhibited effectively by 7-methylguanine in vitro.

The possibility that tRNA: guanine transglycosylase inhibitors are responsible for the Q-hypomodification of tRNA in malignant cells in vivo was suggested by other investigators based on comparable enzyme activities being observed in normal Q-sufficient and malignant Q-deficient tissue. The nature of these putative inhibitors has not been established. However, malignant tissues contain aberrant tRNA methyltransferases, and cancer patients excrete highly elevated levels of tRNA catabolites; especially methylated derivatives. Therefore, our results suggest that increased endogenous methylated purines (e.g. 7-methylguanine) may be involved in inducing the Q-hypomodification of tRNA associated with neoplasia.

The data obtained allowed us to formulate a hypothesis concerning the specific role of altered tRNA metabolism in carcinogenesis, and that hypothesis was presented in the manuscript in press listed below. The hypothesis states that at some early time after the induction of carcinogenesis there is an elevation of tRNA methylation which leads to the increase in the levels of methylated tRNA catabolites. These catabolites then modulate tRNA modification further, either by inhibiting modification (e.g. by blocking tRNA: guanine transglycosylase) or by acting as an alternative substrate. New tRNA isoaccepting species are generated by the changes in macromolecular modification and these species might translate different (e.g. fetal) mRNA's that are present. By this method, onco-developmental proteins would be synthesized. Some of the new proteins could be tRNA methyltransferase, and this would start the cycle over again at a more aberrant level. The cycle could repeat many times with accruing phenotypic changes until the state of neoplastic transformation is reached.

The hypothesis offers an explanation for the staging process of carcinogenesis and perhaps for promotion of carcinogenesis. Experiments are being initiated with the human cells to determine if altering tRNA metabolism influences promotion.

d. Publications

Trewyn, R.W., Elliott, M.S., Glaser, R., and Grever, M. Alterations in tRNA metabolism as markers of neoplastic transformation. II. Biochemical and Biological Markers of Neoplastic Transformation (P. Chandra, ed.) Plenum Publishing Corp., New York, 1982 (in press).

ott, M.S., and Trewyn, R.W. Queuine hypomodification of tRNA induced by
thylguanine. Biochem. Biophys. Res. Commun. (submitted).

Personnel

ld W. Trewyn, Ph.D., Assistant Professor of Physiological Chemistry,
cipal Investigator

S. Elliott, Ph.D. Candidate

A. Davakis, M.S. Candidate

Interactions

International Advanced Study Institute, Corfu Island, Greece; 9/28/81 to
/81. Institute Topic: Biochemical and Biological Markers of Neoplastic
Transformation

Presentation Title: Alterations in tRNA Metabolism as Markers of
Neoplastic Transformation

