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Katedra i Zakład Chemii Ogólnej Akademii Medycznej w Lublinie Kierownik: prof. dr hab. Kazimierz Pasternak

Katedra i Zakład Chemii Fizjologicznej Akademii Medycznej w Lublinie Kierownik: prof. dr hab. Marta Stryjecka–Zimmer

KAZIMIERZ PASTERNAK, STANISŁAWA SZYMONIK–LESIUK, HALINA BRZUSZKIEWICZ–ŻARNOWSKA, TOMASZ BORKOWSKI

tRNA of neoplastic tissues

tRNA tkanek nowotworowych

Transfer RNA (tRNA) has long been recognized as a molecule that participates in the translation of mRNA. Numerous investigator's evidence suggests that tRNA may play a prominent role in the regulation of gene expression (13). In addition, findings in other studies lead to the suggestion that expressed genes, such as regulatory genes, may be regulated in part by tRNA availability. The role of tRNA in growth, differentiation and transformation of the cell is well known. Alteration in tRNA metabolism and modification associated with tissue regeneration and neoplasia has been observed (5, 15, 18). The studies of tRNA structures, modifications and functions in cancer cells were essential in defining the methods of distinguishing cancer cells from normal cells. This is very important in cancer prevention and therapy. This stimulated our interest to compare the tRNA of normal and neoplastic cells.

METHODS

Experiments were carried out on normal liver tissues of 12-week old rabbits of a mixed breed. Uterine myomas were obtained from the Second Gynecology Clinic and malignant cancer (stomach carcinoma) were received after operations from the Municipal Hospital.

Preparations of tRNA from the particular tissues were obtained by phenol-isopropanol extraction according to Sein (19) and Zubay (20). Crude preparations of tRNA were additionally purified by chromatography on DEAE-cellulose column and then subjected to deaminoacylation (7). Contents of tRNA in the investigated tissues were expressed in optical density units by 260 nm. Electrophoretical separation of tRNA was performed in polyacrylamide gel (PAGE) in the first-direction in 10% gel, and the second-direction in 20% gel (9). Preparations of tRNA were dyed in stain-all.

RESULTS

Preparations of tRNA were received from experimental tissues by the method of Sein and Zubay and were separated in one-direction PAGE. As shown on the electrophoretical diagram (Fig. 1), of all experimental tissues, the fractions of 4S region were predominant. In fact, electrophoretical separation of tRNA did not depend on the kind of tissue except for tRNA from carcinoma of the stomach where more light fractions were observed. Also, separation of these preparations in second-direction electrophoresis showed some quantitative differences (various number of spots) as well as qualitative ones (additional spots). The numbers of spots in the separation of tRNA from physiological liver of rabbit was 32 (Fig. 2), tRNA from uterine myoma was 18 (Fig. 3) and tRNA from gastric cancer was 27 (Fig. 4). The quantity of tRNA expressed in the optical density units used in two-dimensional electrophoresis was the same for all investigated tissues. The estimation of the tRNA separation based on the isoacceptors tRNA map from membrane of uterine myoma (Fig. 5) was performed.



Fig. 1. Electrophoretic pattern of tRNA samples on polyarylamide gel; A – rabbit liver tRNA, B – uterine myoma tRNA, C – gastic cancer tRNA

DISCUSSION

Tissue type, and also the state of maturation and differentiation of cells, determinates quantitative as well as qualitative changes of tRNA (3, 4, 10). These changes refer not only to the numbers of the particular tRNA, but also to the numbers of tRNA isoacceptors for different amino acids (17). The existence of quantitative differences in the separation of isoacceptors of tRNA from various organs of the same animals was also stated (11). The results obtained in this paper have revealed the existence of differences in electrophoretical separation of experimental tissues: normal liver, benign tumor (myoma) and malignant tumor (stomach cancer). The numbers of tRNA spots in the electriophoretical diagram of PAGE for particular tissues were various – the most for tRNA from livers of the rabbits (32 spots), the least for tRNA from myoma uteri (18 spots). In the case of tRNA in pathological tissues especially neoplastic ones, is a subject of current experiments (3, 10). It is obvious that total contents of tRNA were increased (1, 6) but the binding ability of amino acids was changed (6, 14, 15) and tRNA specific isoacceptors for pathological tissues appeared and their contents were different



Fig. 2. Two-dimensional electrophoresis of tRNA rabbit liver in polyacrylamide gel



Fig. 3. Two-dimensional electrophoresis of tRNA uterine myoma in polyacrylamide gel

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Fig. 5. tRNA isoacceptors map in human myometrial tissue

(4, 5). Actually, very few papers have appeared dealing with quantitative and qualitative differences of tRNA between normal and pathological contents of human tissues (2, 8, 12, 16). Thorough explanation of our results would be possible after some additional experiments. These would help us to answer if, and what kind of isoacceptors tRNA appear in normal and pathological tissues and what is their amount and scope of modification in these tissues. Hopefully, such evaluation could improve diagnosis and treatment in the future.

CONCLUSIONS

1. The separations of specific tRNAs from neoplastic tissues of human were performed by two-dimensional PAGE.

2. We proved differences in quantities of individual tRNA between tRNA preparations from liver tissue and tRNA preparations from neoplastic tissues.

REFERENCES

- 1. Baranowski W.: Receptor estrogenowy i transportujący kwas rybonukleinowy (tRNA) w mięśniakach macicy u kobiet. Crin. Pol., 57, 660–664, 1986.
- Baranowski W., Tomaszewski J.: Fenyloalanylowe tRNA w tkance mięśniaka macicy kobiety. Gin. Pol., 65, 234–238, 1994.
- 3. Bjork G. et al.: Transfer RNA modifications. Ann. Rev. Biochem., 56, 263–287, 1987.
- Borek E.: Modification of nucleic acids in relation to differentiation. Trends Biodrem. Sci, 2, 36, 1977.
- Borek E., Kerr A.: Atypical transfer RNA's and their origin in neoplastic cells. Adv. Cancer Res., 15, 163–190, 1972.
- 6. Brzuszkiewicz-Żarnowska H. et al.: Aminoacylacja tRNA *in vivo* w mięśniakach macicy. Gin. Pol., 62, 437–440, 1991.
- Denney R.M.: Detection and partial purification of rapidly sedimenting forms of aminoacyltransfer ribonucleic acid synthetases from human placenta. Arch. Biochem. Biophys., 183, 156– -167, 1977.
- 8. Emmerich B. et al.: Relationship of guanine-lacking transfer RNAs to the grade of malignancy in human leukemias and lymphomas. Cancer Res., 44, 3215–3217, 1985.
- 9. Fradin A., Gruhl H.: Mapping of yeast tRNAs by two-dimensional electrophoresis on polyacrylamide gels. FEBS Lett., 50, 185-190, 1975.
- Giege R. et al.: tRNA structure and aminoacylation efficiency. Progr. Nucleuc Acid Res. Mol. Biol., 45, 129–206, 1993.
- 11. Hatfield D., Caitus M.: Specificity of tRNAs in mammalian tissues. Fed. Proc., 28, 349-358, 1969.
- 12. Huang B. et al.: Relationship of the guanine content of transfer ribonucleic acids to histopathological grading and survival in human Jung cancer. Cancer Res., 52, 4696–4700, 1992.
- 13. Okada N. et al.: Detection of unique tRNA species in tumor tissues by *Escherichia coli* guanine insertion enzyme. Proc. Natl. Acad. Sci. USA, 75, 4247-4251, 1978.
- Pasternak K. et al.: The activity of aminoacyl-tRNA synthetases in stomach cancer. Appl. Biol Commun., 3/5-6, 137-142, 1993.
- Pasternak K.: Aktywność akceptorowa tRNA raka sutka. Post. Med. Klin. Dośw. 4, 165–169, 1995.
- Pasternak K. et al.: Efektywność wiązania aminokwasów przez tRNA w raku żołądka. Pamiętniki 56 Zjazdu Chir. Pol., 4, 1461–1465, 1993.
- 17. Persson B.: Modification of tRNA as a regulatory device. Mol. Microbiol., 8, 1011-1016, 1993.
- Randerath K. et al.: tRNA alterations in cancer. Recent Results in Cancer Res., 84, 103–119, 1983.
- 19. Sein K.T. et al.: A simple modified method for the extraction of rat liver sRNA. Anal. Biochem., 28, 65-69, 1969.
- 20. Zubay G.: The isolation and fractionation of soluble ribonucleic acid. J. Mol. Biol., 65, 375-378, 1972.

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STRESZCZENIE

Przeprowadzono badania tRNA otrzymywanych z wątroby zdrowych królików, łagodnych guzów (mięśniak macicy) i guzów złośliwych (rak żołądka). Preparaty tRNA otrzymywano metodą ekstrakcji fenolowej według Seina i Zubaya, a następnie przeprowadzano rozdział elektroforetyczny w żelu poliakrylamidowym, stosując rozdział dwukierunkowy (PAGE). Wykazano różnice zarówno w ilości, jak i rozmieszczeniu plam izoakceptorowych tRNA w poszczególnych rodzajach badanych tkanek.