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Effect of Glucose on the Expression of *c-myc* Gene in Cultured RINm5F Cell

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The study was designed to examine the effect of glucose on the expression of c−myc gene in cultured RINm5F cells. Ater monolayer culture was established in RPMI 1640 media supplemented with 10% fetal calf serum (FCS), the cells were cultured in various concentrations of glucose and 1 or 10% FCS for another 24 hours. A mRNA was extracted from the cultured cells by a single step method, and Northern analysis was done to detcet RNA band.A 0.5 kilobase single band was detected as c−myc mRNA. The expression of c−myc gene mRNA was reduced with increased concentration of glucose with 1% FCS.However, supplementation of 10% FCS abolished the effect of glucose on expression of c−myc gene. These findings suggested that glucose in conjunction with other growth promoting factors played an important role in expression of oncogene and cell growth in RINm5F cells.

Key Words : *c−myc, RINm5F cell, glucose effect*

INTRODUCTION

Recently, attentions have been focused on the role of oncogenes in cell proliferation. Normally found proto−oncogenes seem to play an important role in cell development and growth (Hayward et al, 1981). It was suggested that the process leading from a normal to a fully transformed cell may be really a series of multistep events under the control of different classes of oncogenes (Brodeure, 1984). Cellular oncogene such as *c−myc* is classified as establishment gene, and encodes for a nuclear protein that plays a critical role in the regulation of gene transcription and DNA replication.

In cell culture system, cellular proliferation is promoted by two factors : nutrient supply such as glucose and metabolic regulator such as insulin(Taub et al, 1987). Experiment with rat insulinoma cell, i. e, RINm5F cell, appears to provide a unique model for the study of cell growth because insulin is produced by themselves and the cell function is partially dependent on a glucose. A change of glucose concentration of the culture media of RINm5F cell actively influences growth as well as functional characteristics of the cell. The *c−myc* gene has been known to be actively expressed in rapidly growing cells(Nakamura et al, 1988), but the effect of glucose on the regulation of mRNA trancription is still controversial. An en-

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hanced transcription of *c−myc* gene mRNA in high glucose concentration until 2 hours but inhibited expression after that was reported (Yamashita et al 1988). However, in other human cell line cells reduced expression contradictory to previous report was reported recently by other group(Briata et al, 1989 b). Therefore, the study was designed to assess the role of glucose on the *c−myc* gene expression in RINm5F cell line cells.

MATERIALS AND METHODS

The cell line was derived from a rat islet β−cell, RINm5F. It was established at NIH(Bethesda, Md.)and was kindly provided from Dr. Yoon of Calgary University(Calgary, Canada). The cells actively secreted insulin in culture media and typically were being maintained in RPMI 1640 media with supplementation of 10% fetal calf serum(FCS). After the monolayer culture was established, then the cells were exposed to different concentrations of glucose such as 50,100,200, and 400 mg/dl for next 24 hours at 37℃. The minimal concentration of glucose on culture media for normal growth of RINm5F cell was 50mg/dl. The RINm5F cell was hardly growth on the completely glucose free condition.

To define the effect of serum on *c−myc* gene expression by other than glucose, the FCS was added to the media by 1, or 10%. After cell culture, the cells were collected by a policeman, and a mRNA was prepared by a single-step procedure(Sambrook et al, 1989). The cell pellet was mixed with 1 ml of denaturing solution(4M gunanidium thiocyanate, 25 mM soduim citrate, 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol, pH 7.0) and was homogenized briefly. Then the homogenized solution was mixed with 0.1 ml of 0.2 mM sodium citrate(pH 4.0), 1 ml phenol and 0.2 ml of chloroform−isoamyl alcohol mixture(49 : 1), and was placed on ice for 15 minutes. The mixture was centrifuged at 4℃ at 10,000g for 20 minutes and the supernatant was collected into 1.5ml Eppendorf tube. For precipitation of RNA, 1 ml of isopropanol was added and centrifugation was done at 10,000g for 4℃ for 20 minutes. The precipitated RNA was washed with 70% ethanol and was dried for 15 minutes in a vacuum concentrator. The RNA was dissolved in 50μl of distilled water and was quantified by UV spectrophotometer at 260 nm and 280 nm. The RNA was stored at −70℃ until electrophoresis(EP). The total amount of extracted RNA was 50∼100μg from $6x10^6$ RINm5F cells.

Fifteen micrograms of RNA were denatured with deionized glyoxal at 55℃ for one hour and were used for EP in 1% agarose gel in 10 mM sodium phosphate buffer. EP was performed at 70 volts for 4 hours with a change of the buffer by 1 hours. After the EP, RNA was transferred to a nylon membrane paper.

The Northern blot hybridization technique was used for detection of RNA band. The *c−myc* gene Probe was 8.4kb genomic DNA (American Type cultue Collection no. 41010) and was inserted into the pBR 322 vector at the Eco RI and Hind Ⅲ cloning sites. Hb101 was used as the host of the cloned vector. ^{32}P labelling of *c−myc* gene probe was performed by random hexamer method.

Hybridization of the nylon membrane with a labeled DNA probe was performed with hybridization solution(0.5 M sodium phosphate and EDTA solution, 30% formamide, 1% bovine serum albumin, 5% sodium dodesyl sulfate, and 250μg salmon sperm DNA in a sealed vinyl bag at 50℃ for 12-16 hours. Stringent Washing of the nylon membrane was performed and autoradiography procedure was done. The intensity of *c−myc* gene RNA band was analyzed by scanning densitometer (GS300 Hoefer Scientific Inst., SanFrancisco CA) and expressed as relative absorbances in arbitary units.

Strip−wash of the membrane was done in boiling water and it was reused for hybridization with the chicken β actin probe(Oncor, Gaithersburg, Md).

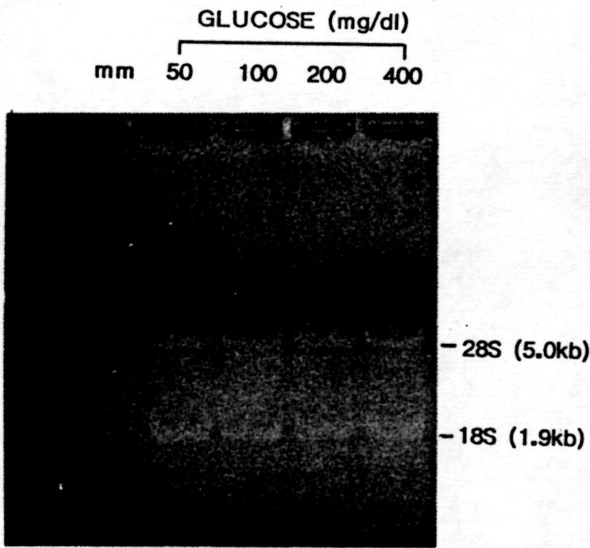

Fig. 1.

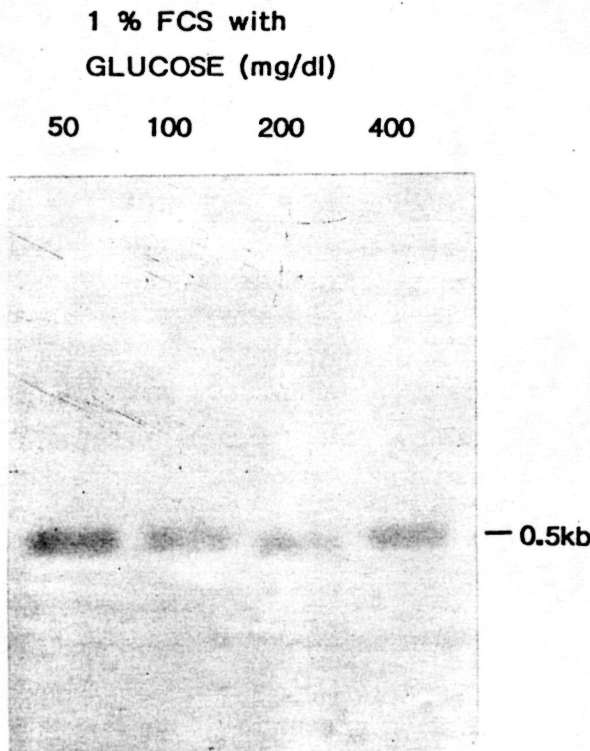

Fig. 2.

RESULTS

About 15μg of total RNA were used for formamide—agarose elecrophoresis. Intact 18s and 28s RNA band were clearly showed

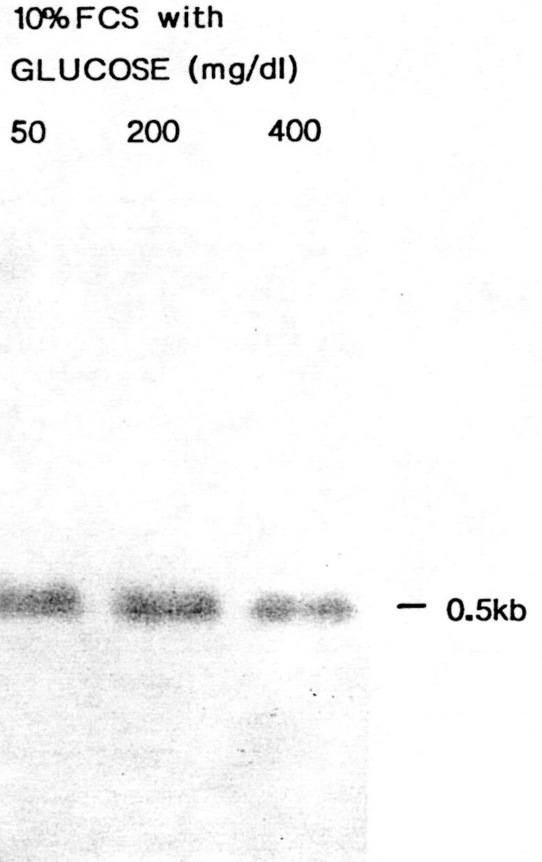

Fig. 3.

with ethidium bromide staining(Fig. 1). Major RNA band of 0.5 kb size was identified by hybridization of *c—myc* gene probe. Even though this size was smaller than that of the hybridization with the *v—myc* DNA probe, it was compatible with that of previous report on truncated transcrption (Rabbitts, 1985). In culture condition from the low to the high glucose concentration with 1% FCS for 24 hours culture, the maximal expression of *c—myc* gene mRNA was noted in 50mg/dl glucose while *c—myc* mRNA expression was reduced with an increase of glucose concentration (Fig.2). The relative absorbances of *c-myc* transcript(in arbitary units)were 54.3 $\pm$2.4, 48. $\pm$12.3, 23.3$\pm$8.4, and 20. 1$\pm$11.2 respectively in 50,100,200,400mg/dl glucose concentration. However, at 10% FCS, high glucose did not affect the degree of *c—myc* gene transcription (Fig. 3). The β—actin gene expression was not

altered by exposure to different glucose concentrations.

DISCUSSION

The *myc* oncogene was originally observed in the genomes of a group of acutely transforming retroviruses which can induce a variety of neoplastic diseases in birds. The viral *v−myc* gene encodes a single polypeptide of 110 k Da and aminotermninal half of this protein is specified by retroviral gag gene. Cellular homology of *v-myc* has been observed in chicken as well as mammalian genomes. A sequence of ellular *c−myc* gene shares homology with *v−myc* gene in two exons.

The steady state mRNA levels of *c−myc* increase when cells are stimulated to proliferate, and the expression no longer occurs at nondividing cells(Campisi et al, 1984). The expression of *c−myc* gene may be specific to the G_1 phase of cell cycle but the cellular level of *c−myc* mRNA is constant through the cell cycle(Thompson et al, 1985).

The RINm5F cell was derived from the rat pancreatic islet cell and had various kinds of proto−and cellular−oncogenes. This cells secrete rat insulin on glucose stimulation. Glucose is a essential energy source in the cell culture system(Sener et al, 1987), this cell line was used for the model of proto−oncogene expression studies.

Since there are two RNA start sites, two main mRNA species are derived from the normal *c−myc* gene. Recent study on the stability of *c−myc* mRNA in transformed cells expressing *c−myc* gene shows that truncated myc mRNA is more stable than its normal counterpart(Rabbitts, 1985). This result means that exon 1 plays a post−transcriptional role in the process of *c−myc* mRNA degradation in vivo, and truncation of *c−myc* gene has a crucial role in tumorigenic pathogenesis(Keath et al, 1984). In our experiment, we used the transformed rat insulinoma cell and adopted very low serum condition for cell culture. Therefore, it is easy to expect the expression of truncated, rather small sized *c−myc* gene mRNA to maintain tumorous condition of cell.

c−myc gene mRNA expression was increased with short−term 2 hour exposure ; however, the expression was reduced with long−term 24 hour exposure(Yamashita et al, 1988). In the other report using other cell line, *c−myc* gene expression was decreased 3∼4 times in high glucose concentration as compared to low glucose concentraion(Briata et al, 1989 b). This was compatible to our result. Decreased *c−myc* mRNA transcription to high glucose was noted in the culture condition of 1% supplementation of FCS ; however, this effect was aboished by 10% FCS. The concentration of FCS seems to be explained by the effect of the various kinds of growth factors in the FCS on the exression of the oncogenes.

This finding suggested that proper concentration of FCS would be necessary for the evaluation of glucose or other growth factor effects on the expression of oncogenes. The decreased *c−myc* gene expression in high glucose condition was also observed from endothelial cells(Cagliero et al, 1988). Regarding insulin receptor gene expression of cultured cell, as contrary to *c−myc* gene, the high glucose enhanced expression of insulin receptor gene (Braita et al, 1989 a)

Addition of glucose to the culture media stimulated the growth of rat insulinoma cells. The mechanism of signal transduction to the insulin receptor gene may be different from that of the *c-myc* gene. Our result suggested that differential regulation of growth promoting related genes by different glucose concentration was belived to be an urgent point to be solved. Base on these data, a kind of tumor cell would be relatively insensitive to glucose and insulin induced hypoglycemia in the insulinoma or other tumors may exert enhancement of tumor growth. However it might be necessary to study further on the stimula-

tory effect of the low glucose concentration on tumor growth to extrapolate these resuts to the in vivo system.

REFERENCES

Briata P, Gherzi R, Adezati L, Cordera R : *Effect of two different glucose concentrations on insulin receptor mRNA levels in human hepatoma Hep G_2 cells. Biochem Biophys Res Commun 160 : 1415–1420, 1989.*

Briata P, Laurino C, Gherzi R : *c–myc gene expression in human cells is controlled by glucose. Biochem Biophys Res Commun 165 : 1123–1129, 1989.*

Brodeur GM:*Amplification of the N-myc in untreated human neuroblastomas correlates with advanced disease stages. Science 224 : 1121–1124, 1984.*

Cagliero E, Maiello M, Boeri D. Roy S, Lorenzi M : Increased expression of basement membrane components in human endothelial cells cultured in high glucose. J Clini Invest 82 : 735–738, 1988.

Campisi J, Gray HE, Pardee AB, Dean M, Sonenshein GE : *Cell cycle control of c–myc but not c–ras expression is lost following chemical transformation. Cell 36 : 241–247, 1984.*

Dani CH,, Blanchard JM, Piechaczyk, M, Sabouty SE, Marty L, Jeanteur PH : Extreme instability of myc mRNA in normal and transformed human cells. Proc Natl Acas Sci USA 81 : 7046–7050, 1984.

Hann SR, Thompson CB, Eiseman RN : *c–myc oncogene protein synthesis is dependent of the cell cycle in human and avian cells. Nature 314 : 366–369, 1985.*

Hayward WS, Neel BG, Astrin SM : *Activation of a cellular oncogene by promotor insertion in ALV–induced lymphoid leukosis. Nature 290 : 475–479, 1981.*

Keath EJ, Caimi PG, Cole MD : *Fibroblast lines expressing activated c–myc oncogenes tumorigenic in nude mice and syngeneic animals. Cell 39 : 339–348, 1984.*

Nakamura KD, Turturro A, Hart RW : *Elevated c–myc expression in progeria fibroblast. Biochem Biophys Res Commun 155 : 996–1000, 1988.*

Praz GA, Halban PA, Wollheim CB, Blondel B, Strauss AJ, Renold AE : *Regulation of immunoreactive insulin release from a rat cell line(RINm5F). Biochem J 210 : 345–352, 1983.*

Rabbitts PH : *Truncation of exon 1 from the c–myc gene results in prolonged c–myc . mRNA stability. EMBO J 4 : 3727–3733, 1985.*

Saito H : *Activation of c–myc gene by translocation ; A model for translation control. Proc Natl Acad Sci USA 80 : 74476–7480, 1983.*

Sambrook J, Fritsch EF, Maniatis T : *Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, New York, 1989.*

Sener A, Malaisse WG : *Stimulation by D–glucose mitochondrial oxidative enents in islet cells. Biochem J 246 : 89–95, 1987.*

Slamon DJ, de Kernion JB, Verma IM, Cline MJ : *Expression of cellular oncogenes in human malignancies. Science 224 : 256–262, 1984.*

Taub R, Roy A, Dieter R, Koontz J : *Insulin as a growth factor in rat hepatoma cells ; Stimulation of proto–oncogene expression. J Biol Chem 262 : 10893–10897, 1987.*

Thompson CB, Challoner PB, Nieman PE, Groudine M : *Levels of c–myc oncogene mRNA are invarient through the cell cycle. Nature 314 : 363–366, 1985.*

Yamashita S, Tobinaga T, Ashizawa K, Nagayama Y, Yokota A, Harakawa S, Inoue S, Hiraya H, Izumi M, Nagataki S : *Glucose stimulation of protooncogene expression and deoxyribonucleic acid synthesis in rat islet cell line. Endocrinology 123 : 1825–1829, 1988.*