THE ROLE OF FIBRONECTIN AND COLLAGEN CELL ADHESION MECHANISM

(The Role of extracellular matrices in adhesion of cells in vivo and in vitro)

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Introduction

The adhesion of cells during cultivation in vitro /glass, plastic, biologically active substrates etc./ is considered by the majority of research workers as a division of theoretical and applied biology, which is closely connected with such subdivisions as embryogenesis, wound healing, surgical injury, carcinogenesis /metastatic spreading of tumor cells/, gulding morphogenesis, cell cycles, cell movements, control /measurement/ of in vitro cell growth [4,7,9,10].

The central role of organizers and promoters of cell – substrate relations is played by the family of adhesive glycoproteins, i.e. fibronectin, laminin, vitronectin, trombospondin and others. The processes taking place during the interaction and movement of cells are reflected in their functions and morphologic structuring. These effects can be different depending on either prior or logically built models. The basic conception we are developing through our research is shown in Fig.1.

We have chosen the following models as the subjects of our research: regenerating rat liver tissue with partial and total hepatectomy; and cultivated animal cells. We were considering the first model as mostly synchronized proliferation of hepatocytes in vivo and the second one – as the model to reflect signals from microenvironment and its realisation in the form of specific morphological and functional manifestations in vitro. The main factor, which is uniting these models, is the specific cell adhesion to the substrates of microenvironment.

The specific cell adhesion is a dynamic interaction process of cell receptors with ligands and the substrate of extracellular matrix /ECM/ expressed in premitotic period. Successive processes of the interaction consist of attachment, spreading, adaptation/growth and development of intracellular structures/proliferation, differentiation and deadhesion [10].

Research methods

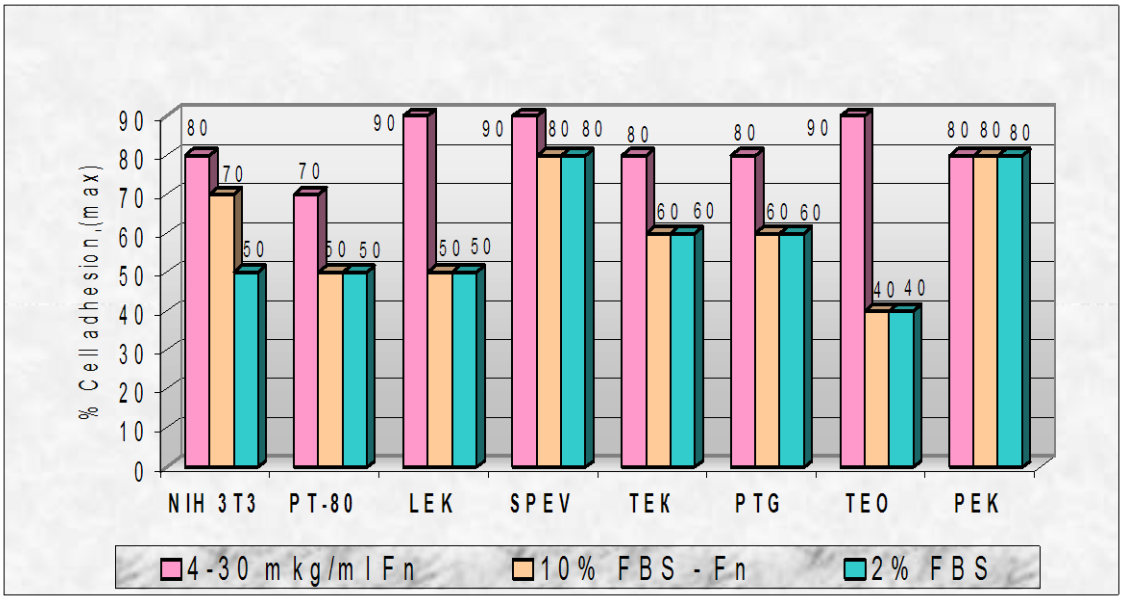
Male WISTAR rats, aged 1-2 months have gone through subtotal and partial hepatectomy. They were operated on with general anesthesia /ethyl ester/ with the observance of all antiseptic rules. Rats with experimental laparectomy were used as control animals. The level of blood plasma fibronectin was estimated by turbidimetric method of Boehringer-Ingelheim test system [see Instruction of This Firm]. The activity of a1AT and a2MG proteinase inhibitors was measured by a regular method [11]. The cell lines were obtained from the VIEV Cell Cultures Collection and cryobank /embryo pig kidney /SPEV/, calf kidney /PT-80/, pig thyroid gland /PTG/, bovine embryo lungs /LEK/, calf testicle /epithelium-like/ cells /TEK/, bovine embryo kidneys /PEK/ together with NIH-3T3 from the Collection of Russian Academy of Sciences was carried out on the Egla MEM and F-12 media with the addition of bovis fetal serum /1-2%/ and some additives: transferin, insulin, thyrotropic hormone and fibroblast growth factors FGF, fibronectin, and type I, III, IY collagens. In a range of experiments with the use of fetal serum of high concentration fibronectin exhausted by the affine chromatography method on gelatin-agarose /Sigma/ fetal serum was used as a control substance. Morphometric indicators were estimated with the help of "Leitz TAS-plus system "/Germany/ with the software of the same company plus some of our own programmes. We took into consideration the size of the cells, their quantity, the spreading area, nucleus and cytoplasmic correlation etc. The cell adhesives was estimated according to the [2] method.

The results of the experiments

In a number of experiments with partial hepatectomy we have found out that after 48 hours of the experiments the level of fibronectin in rat blood plasma reaches 500% of its normal concentration and after 72 hours its level reduces to the control figures. At the same time the indicators of antitryptic blood activity are undergoing the same changes [11]. Taking into consideration phase changes of the investigated indicators and positive correlation between the level of fibronectin in blood plasma and antitryptic blood activity /$p = 0,66$ with $p < 0,55$/ we could assume that fibronectin normally circulating in blood is characterized by low adhesiveness and opsonic activity, but if due to different reasons the balance of protease inhibitors

reduces, the activation of fibronectin molecules and their opsonic activity as well as the formation of biologically active fibronectin fragments is possible [8]. After a number of experiments on rats with partial hepatectomy and simultaneous blocking of the reticuloendothelial system by the intraperitoneal injection of the trypan blue we found out that synthesis of fibronectin is 80% dependent on hepatocytes. It is evident that the "inducing signal" of the generation of soluble fibronectin is received by hepatocytes. We hypotetically assumed the existence of paracrinic mechanism to regulate the generation of soluble fibronectin by hepatocytes.

Cultivating cells on the biologically active foundations and introducing in them albumin, gelatin /collagens/ and also fibronectin and its total papain fragment we have shown the increase of adhesive cell activity to substrates in accordance with the decrease of the percentage of fetal bovine serum /FBS/ from 10 to 2% in comparison with the similar experiments with the use of fibronectin exhausted serum. In case of cultivation without serum with introduction of 2 to 100 microgram to 1 ml of a medium we obtained an exponential curve for each cell line, which enables us to establish the maximum of adhesive potential and optimum concentrations of exogenous fibronectin for each cell line [2,3].



Summarized results of experimental data on research of adhesive properties of biologically active foundations are shown in Fig.2. From the given data it is clear that the adhesive spectrum for the majority of cell lines in comparison with the test line

accounts for 35-85%. The reduction of FBS to 2% with the introduction of exogenous fibronectin in optimum quantities doesn't reduce the adhesive activity in comparison with the medium with 10% of fibronectin. The introduction of exogenous fibronectin in optimal concentrations of 4 to 30 microgram/ml of a medium to media without serum increases cell lines adhesiveness by about 30% in comparison with the control line. The mentioned properties can be called weak in SPEV cells, which has lost its capacity to have substrate-dependent connections due to long cultivation; the same is true of PEK cells in which this kind of relationships are not realized because of the reasons which are not yet clear. In this connection we have carried out a number of experiments with the lines, which have phenotypically similar properties, from the ATSS bank: MDBK lien. We'd like to mention a high level of down-regulation in LEK and TY diploid cell lines. Most probably intercellular inductive potential and residual hormone dependence that are less vivid in other lines are being displayed here.

We have also obtained some data on growth characteristics, i.e. planting concentration of cells /density/, the period of monolayer forming, proliferation index. Generalized analysis of randomized data is given in Table 1.

Table 1. Growth and build-up of cells on BAS till another entwine.

Foundation	Cell Culture	Planting concentration: cell/ml x 10^4	Monolayer forming /days/	Proliferation index
BAS /5-30	TR	8,0	2-3	3,8
mcgr/cm2	SPEV	5,5	3	6,2
sFn/	PTG	5,0	3-4	3,9
(n=8)	PT-80	6,6	3	3,3
Control	TR	8,0	3-4	2,8
/plastic	SPEV	5,2	3	4,8
"Falcon"/	PTG	5,0	3-4	3,2
(n=6)	PT-80	6,4	3-4	2,7

From the given data it is clear that the increase of the cell mass build-up on the basis of biologically active foundations in comparison with the control line accounts for 10-15% and in case of high density cultures it sometimes accounts for 30% in comparison with the control cell lines grown on plastic. The obtained data estimated in accordance with the conception being worked out proves the assumption that the processes taking place on the border of the compartment: cell-matrix are decisive in the forming of specific microenvironment of cells and the appropriate realization of their morphological characteristics. Further development of other parameters for the conditions of optimal cultivation of each cell line can result in complete mechanism of guided growth of normal differentiated cells including transgene ones.

REFERENCES :

[1] AKIJAMA,S.K., YAMADA,S.S., CHEN,W.-T. And YAMADA , K.M.: J. Cell Biol.109,863-875 (1989)

[2] BARNES, S.D., SATO,G.: Anal. Biochem. 102, 255 – 270 (1980)

[3] BELL,G.J: -Science 200,4342,618-627 (1978)

[4] EDELMAN,G.M.: Anal. Rev. Cell Biol. 129,1707 – 11720 (1995)

[5] ECKES,B., MAUCH,C., HUPPE,G. And KRIEG,T.: FEBS Lett. 318, 129 – 133 (1993)

[6] ELSDALE,T. And BARD,J.: J. Cell Biol. 54, 626 – 637 (1972)

[7] JOHANSSON,S., SWINENG,G., WENERBERG,K.., ARMULIK,A., and LOHIKANGAS, L.: Front. In Bios 2, March 1,126 – 146 (1997)

[8] SAVADA,N., TAMURA,C.A. and SATLER ,G.L.: Exp. Cell Res.167, 458-470 (1986)

[9] TOTOWA,N.J.:ANIMAL CELL BIOTECHNOLOGY: methods and

protocols, Human Press, 302p (1999)

[10] FEDOTOVA,A.V., SNEZKO,A.G. and FEDOTOV, A.V.: Biotechnology /Mos./ 4, 15 - 17 (1992).

[11] OVCHARUK,I.N.,FEDOTOV,A.V. and FEDOROV N.A.: Medical Problems of The Biochemysrty. Kemerovo, 46 - 47 (1985)

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