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Selective cytotoxicity and modification activity of picornaviruses on transformed cell lines

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Aim. *We do analyze of the dynamics of morphometabolic changes in transformed cells (of susceptoible lines) demonstrating resistance to picrnaviral infection.* ***Methods***. *The study was performed by application of cell culture technology and a complex of cytochemical and cytophotometric assays. Were used picornaviruses from various genu.* ***Results***. *According to the results obtained, resistant to picornavirus infection cells of different susceptible lines have similar changes in the phenotype. They have decreased number of nucleoli and increased percentage of euploidy (and near euploid). In resistant cells of all cultures the reduction in amount of DNA and RNA both in nucleus and in cytoplasm was found. All these data correlated with the increased euploidy (and near euploid) of the resistant population. All picornavirus resistant cells had a less transformed phenotype, and decreased proliferative activity. Decreased nucleolar status becomes apparent by reduction of all nucleolar indices.* ***Conclusions***. *Picornaviruses on the susceptible cells produce 2 types of changes – selection and modification. Whatever the mechanism, it is specific for an individual virus, since no restrictions occur in case of infection caused by another picornavirus.*

Keywords: picornavirus, euploidy, nucleus, nucleolus.

Introduction. Although selection for host cells that resist viral infection during picornaviral persistence has been reported, their role in persistence has not been elucidated, and the basis for host cell resistance is not clear [1, 2]. We analyze the possibility of complex metabolism changes in resistant cells as a protecting factor against virus infection.

Aim of this research is the analysis of the dynamics of morphometabolic peculiarities of the becoming resistant cells as well as the comparison of phenotype of picornavirus resistant cell of sensitive to the cytotoxic activity lines.

Materials and methods. *Cells*. HEp-2, HEK293, Caco-2, BHK cells, continuous transformed cultures, were cultivated as described (ATCC).

Viruses. EMCV (Columbia-SK strain) was used at multiplicity of infection 0.1 TCD_{50} per cell. Poliovirus-1 /Sabin/: was used at multiplicity of infection 0.1 TCD_{50} per cell. Foot-and-mouse disease virus (strains O NKR-194; A-22; Asia-1 – FMDV-O, FMDV-A, FMDV-Asia) was used at multiplicity of infection 0.1 TCD_{50} per cell. Viral titers were calculated by the method of Karber. As a control the parallel conducted passages of noninfected cultures were used.

After lytic viral infection in some flaks were present isolated cells which have resistance against corresponding virus. Resistant cells were received by one time infection.

The second method of receiving the resistant cells was described by Taber et al. [3]. Chronic viral infection was received by one time infection of 48-hour monolayers of cultures CaCo-2 and HEp-2 by poliovirus-1 /Sabin/. The multiplicity of infection was 0.000001 TCD_{50} per cell. Infected cells were incubated at 36.5–37 °C. After 5–11 passages some cultures become free from virus and cells received resistance to a corresponding virus. Chronic infections were repeated 3 times and summarized data were presented.

Table 1
Amount of DNA in control, resistant cells of the RD, Hep-2, HEK293, HeLa, CaCo-2, BHK-21 cultures («c» units)

Cultures	Control «c»	EMCV acute infection «c»	Poliovirus acute infection «c»	Poliovirus chronic infection «c»	FMDV-O acute infection «c»	FMDV-A acute infection «c»	FMDV-Asia acute infection «c»
RD	3.9 ± 0.6	2.5 ± 0.2*	–	–	–	–	–
Hep-2	4.4 ± 0.4	3.4 ± 0.2*	3.8 ± 0.2*	3.6 ± 0.1*	–	–	–
HEK293	5.9 ± 0.8	4.1 ± 0.5**	–	–	–	–	–
HeLa	5.7 ± 0.8	4.6 ± 0.8	4.0 ± 0.4**	–	–	–	–
CaCo-2	5.6 ± 1.0	–	3.2 ± 0.7	4.1 ± 0.4	–	–	–
BHK-21	2.8 ± 0.4	–	–	–	2.0 ± 0.8	3.0 ± 0.2	1.8 ± 0.1*

*Significant compared to control, $p < 0.05$–$p < 0.01$; **$t = 1.88$; $t = 1.89$.

Cytochemical and cytophotometric analysis. For simultaneously staining DNA and RNA the methyl green, pyronin Y method was used. DNA quantification was done by Feulgen staining. For quantification of RNA was used gallocyanin chromalum stain. In gallocyanin chromalum preparation the control data were defined as 100 %.

Cell viability test. Was used the trypan blue exclusion test, cells were incubated for 5 min with 0.04 % trypan blue («Sigma», USA).

Results and discussion. The received chronically infected cultures were characterized by insular and slower growth (approximately twice), lack of the capacity to form monolayer and constant decreased production of a virus. After 5–11 passages some cultures became free from poliovirus and cells received resistance to it. In microscopic examination of chronically infected cultures we revealed a gradual change towards small round cell morphology.

The received resistant cells survived under the infection with analogical virus, but did not survive under the infection with other viruses.

Proliferative activity of the resistant to picornavirus cells of susceptible cultures was lower than that of the cells of control populations. It happens due to about 2–3-fold increase of the interphase of mitotic cycle compared to the control populations.

According to the results of cytophotometric analysis of the acute picornaviral infection of Hela, CaCo-2, HEK293, RD and HEp-2 cultures had similar morphological changes. The survived cells had similar phenotypical characteristics. This was evidenced by the presence of binucleate cells, and the decreased number of nucleoli in each nucleus, which is visible using MGP staining. Those cells were smaller and had a round shape, in comparison with the control ones. The reduction in sizes applies to both the cells and theirs nuclei. The resistant cells had significantly decreased DNA amount (Table 1). The similar changes were noticed under the influence of chronical picornaviral infection.

The nucleoli number in investigated cells was reduced compared to the control population, which testifies the oppression of translational activity (Table 2).

The percent of euploid cells among the resistant cells in RD, Hep-2, HEK293, BHK-21 and HeLa, significantly increased under the acute infection of EMCV. In case of an acute and chronic poliovirus infection among the resistant cells in Hep-2 cells the percentage of euploid cells also significantly increased, whereas in the CaCo-2 cells such changes are insignificant (Table 3). The disparity in viral effects observed in CaCo-2 cells can be explained by diverse levels of differentiation [4].

It can be noticed that in all cases of resistant cells, the number of nucleoli in the nuclei has tendency to decrease. This data correlates with the increased percentage of euploid cells in resistant populations. The reduction in the nucleoli number testifies oppression of metabolic activity in common and translational activity in particular.

The structure of euploid population also changed. In control population of CaCo-2, RD and Hep-2 cells prevailed over the tetraploid cells with a few number of octaploid and 16 «c» cells. In resistant to picornavirus cells appeared a great deal of diploid cells and disappeared the population of 8 «c» and 16 «c» cells. In resistant HEK293 cells the tetraploid population increased, the octapoid disappeared and in HeLa aroused diploid cells.

In resistant cells of all cultures is found the reduction of DNA, RNA amount, in both nucleus and cyto-

Table 2
Changes in nucleoli number in control and resistant cells of the RD, Hep-2, HEK293, HeLa, CaCo-2 cultures

Cultures	Control	EMCV acute infection	Poliovirus acute infection	Poliovirus chronic infection	FMDV-O acute infection	FMDV-A acute infection	FMDV-Asia acute infection
RD	2.7 ± 0.3	1.6 ± 0.1*	–	–	–	–	–
Hep-2	2.6 ± 0.2	1.3 ± 0.2*	1.6 ± 0.1*	1.5 ± 0.1*	–	–	–
HEK293	2.3 ± 0.2	2.0 ± 0.1	–	–	–	–	–
HeLa	2.5 ± 0.2	1.9 ± 0.2*	2.0 ± 0.2	–	–	–	–
CaCo-2	2.9 ± 0.3	–	2.1 ± 0.1*	2.0 ± 0.2*	–	–	–
BHK-21	2.1 ± 0.2	–	–	–	1.1 ± 0.1*	1.1 ± 0.1*	0.9 ± 0.1*

*Significant compared to control, $p < 0.05$–$p < 0.01$.

Table 3
Euploidy in control and resistant cells of the RD, Hep-2, HEK293, HeLa, CaCo-2, BHK-21 cultures (%)

Cultures	Control, %	EMCV acute infection, %	Poliovirus acute infection, %	Poliovirus chronic infection, %	FMDV-O acute infection	FMDV-A acute infection, %	FMDV-Asia acute infection, %
RD	20.4 ± 3.5	35.4 ± 5.9*	–	–	–	–	–
Hep-2	13.0 ± 4.1	24.2 ± 3.8*	31.0 ± 7.7*	34.0 ± 5.1*	–	–	–
HEK293	26.8 ± 3.8	42.1 ± 4.3*	–	–	–	–	–
HeLa	23.6 ± 3.2	35.5 ± 5.8	32.8 ± 3.3*	–	–	–	–
CaCo-2	50.1 ± 4.1	–	48.2 ± 6.8	57.5 ± 7.3	–	–	–
BHK-21	22.0 ± 5.2	–	–	–	33.0 ± 4.9	46.2 ± 8.7	42.8 ± 6.0

*Significant compared to control, $p < 0.05$–$p < 0.01$.

plasm. All these data correlated with the increased euploidy of the resistant population. In the meantime, the percentage of euploidy in a cell culture at control and viral infection is closely invert correlated to the number of nucleoli. So resistant cells of all cell lines such as HEK293, RD, HeLa, HEp-2, showed decreased number of nucleoli and increased percentage of euploidy. The percentage of euploid cells resistant to the picornaviral infection increases in all cultures (except CaCo-2).

We can also conclude that picornaviruses not only do selective action but also can modify the cells. Modification consisted of the occurrence in survived cells (RD, Hep-2) of diploid population absent in control. In favour of modification testify the changes of cell phenotype that have survived after an acute and chronic infection of picornaviruses. The main selective factor is apoptosis induced in the infected cells. Modification of cells occurs by deblocking the cells in phase G_2 and by stimulation of their division. In general, the phenotype of resistant cells can be characterized as less transformed compared with the intact cell populations.

Resuming the action of picornaviruses on the susceptible cells we can define 2 types of changes – selection and modification. Possibly, the viruses induced apoptosis selectively in multinucleolar aneuploid cells. In favour of this assumption testify the data obtained by Taylor, Martin-DeLeon [5] that the number of nucleolar-forming regions is genotypically determined, so differences among subgroups of multinucleolar cells are more likely than the production of new clones. By Labadie et al. [6] was shown that CaCo-2 cells which are partially resistant to poliovirus induced apoptosis can be selected during persistent virus infection. Castedo et al. [7] described that diploid cells were more stable to the apoptotic influence.

Although the presence or absence of virus receptors on the cell surface remains a major determining factor of the susceptibility of a cell to virus infection, there is now increasing evidence that the intracellular environment plays an important role in the outcome of viral invasion [8].

Important metabolic characteristic of resistant to picornavirus infection cells is a significant decrease in cel-

lular RNA amount. This phenomenon is described in all investigated parts of cells – nucleoli, nucleus, cytoplasm. Undoubtedly first of all the synthesis of rRNA, the major part of total cellular RNA, is oppressed. All picornavirus resistant cells had a less transformed phenotype, such as decreased proliferative activity, reduced DNA amount, increased euploid population and decreased nucleolar status. Decreased nucleolar status becomes apparent by reduction of absolute and relative nucleolar indices. Consequently the viral titers reduction in resistant cells could be the direct result of diminished activity of the RNA synthesis machinery. Whatever the mechanism, it is specific for individual virus since no restrictions occur in infections with other picornaviruses.

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Селективна цитотоксичність і модифікуюча дія пікорнавірусів на трансформованих клітинних лініях

Резюме

Мета. *Мета даної роботи полягала у вивченні динаміки морфологічних і фізіологічних змін трансформованих клітин, резистентних до пікорнавірусної інфекції.* ***Методи***. *Дослідження виконано за умов in vitro із застосуванням цитохімічного і цитофотометричного аналізу. У роботі використано пікорнавіруси різних родів.* ***Результати***. *Встановлено, що стійкі до інфікування пікорнавірусами клітини різних чутливих ліній набувають аналогічних змін у фенотипі. У резистентних клітинах усіх культур як у ядрі, так і цитоплазмі виявлено зниження вмісту ДНК і РНК. Усі ці дані корелюють з підвищенням еуплоїдної (та біляеуплоїдної) популяції за формування резистентності. Усі резистентні до пікорнавірусів клітини були меншими за розмірами порівняно з початковим трансформованим фенотипом та демонстрували зниження проліферативної активності. Зменшення активності ядерець супроводжується вірогідним падінням усіх ядерцевих показників.* ***Висновки.*** *Пікорнавіруси проявляють подвійну дію на чутливі клітини, яка виражається у селективній цитотоксичності і модифікуючому впливі. При цьому механізми їхньої дії є специфічними для кожного окремого пікорнавірусу. Так, резистентні стосовно одного пікорнавірусу клітини виявляються нестійкими до інфекції, спричиненої іншими пікорнавірусами.*

Ключові слова: пікорнавіруси, еуплоїдія, ядро, ядерце.

З. А. Каралян, Г. Р. Авагян

Селективная цитотоксичность и модифицирующее действие пикорнавирусов на трансформированных клеточных линиях

Резюме

Цель. *Целью данной работы явилось изучение динамики морфологических и физиологических изменений трансформированных клеток, резистентных к пикорнавирусной инфекции.* ***Методы***. *Исследование проведено в условиях in vitro с применением цитохимического и цитофотометрического анализов. В работе использованы пикорнавирусы различных родов.* ***Результаты***. *Установлено, что устойчивые к инфицированию пикорнавирусами клетки различных чувствительных линий приобретают аналогичные изменения в фенотипе. В резистентных клетках всех культур как в ядре, так и цитоплазме выявлено снижение содержания ДНК и РНК. Все эти данные коррелируют с повышением эуплоидной (и околоэуплоидной) популяции при формировании резистентности. Все резистентные к пикорнавирусам клетки были меньше по размерам в сравнении с начальным трансформированным фенотипом и демонстрировали снижение пролиферативной активности. Уменьшение ядрышковой активности сопровождается достоверным падением всех ядрышковых показателей.* ***Выводы***. *Пикорнавирусы проявляют двойное действие на чувствительные клетки, выражающееся в селективной цитотоксичности и модифицирующем влиянии. При этом механизмы их действия являются специфичными для каждого отдельного пикорнавируса. Так, резистентные в отношении одного пикорнавируса клетки оказываются неустойчивыми к инфекции, вызванной другими пикорнавирусами.*

Ключевые слова: пикорнавирусы, эуплоидия, ядро, ядрышко.

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