DOI: 10.29326/2304-196X-2023-12-1-13-22

Biological, cytomorphological and karyological heterogeneity of transformed cell lines derived from domestic pig (*Sus scrofa* L.) organs

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SUMMARY

The main advantage of transformed cell lines as compared to primary ones is that they allow generation of the stable material suitable for long-term research and practical use. Therefore, development of new continuous cell cultures from various animal tissues is of great practical importance. Results of examination of transformed cell lines derived from organs of domestic pigs (*Sus scrofa* L.) for their biological, cytomorphological and karyological features are described in the paper. The said cell cultures are confirmed to be susceptible to various animal viruses. Also, a procedure for preparation of new diploid cell culture from porcine spleen (SSs – *Spleen Sus scrofa*) is described. Based on the obtained data analysis it was concluded that the epithelial cells derived from trypsinized porcine spleens could be successfully immortalized. All transformed cell lines of porcine origin have similar morphology with predominated epithelium-like forms. Some of them – SPEV, A_4C_2, RSK – tend to adopt a spherical shape in suspension. Such cell lines as PSGK-30 and PPES cell lines form partial multilayer or they are characterized by significant monolayer compaction with pseudosyncytium formation. Only pseudodiploid cell culture (SPEV cell culture) tends to grow in suspension, it also grows in rotating culture flasks. Karyological transformations in different cell cultures stabilized at certain level. Spontaneous increase in chromosome numbers in the main population of transformed cell lines towards triploidy resulted in stabilization of culture properties and increase in proliferation. PSGK-30 cell culture has the highest modal class – 64 chromosomes. Near-diploid cultures (A_4C_2, RSK) demonstrate stable growth properties and are similar to SPEV cell culture in adopting spherical cell forms in medium, monolayer character and cell morphology. PK-15 cell culture having a distinct karyotype under different cultivation conditions while retaining other culture properties is found to be the most adaptive. A new transformed diploid SSs cell culture is developed by long-term incubation, subcultivation (more than 80 passages) and selection at the FGBI "ARRIAH" laboratory; it can remain diploid or may spontaneously become heteroploid-immortalized during further passaging. The cell hyperploidy is very likely to enhance telomerase activity, which in turn stabilizes immortalization and results in proliferative activity increase. The cell viability has been maintained so far by regular reseedings (split ratio – 1:2–1:3) performed 1–2 times a week.

Keywords: transformed cell line, continuous cell line, hybridoma, diploidy, heteroploidy, modal class of cells, proliferative activity

Acknowledgements: This work was funded by the FGBI "ARRIAH" within the scope of research activities "Veterinary Welfare".

For citation: Manin B. L., Trofimova E. A., Gavrilova V. L., Puzankova O. S. Biological, cytomorphological and karyological heterogeneity of transformed cell lines derived from domestic pig (*Sus scrof*a L.) organs. *Veterinary Science Today*. 2023; 12 (1): 13–22. DOI: 10.29326/2304-196X-2023-12-1-13-22.

Conflict of interest: The authors declare no conflict of interest.

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УДК 619:576.535:636.4:57.082.26

Биологическая, цитоморфологическая и кариологическая гетерогенность постоянных линий клеток, полученных из органов домашней свиньи (*Sus scrofa* L.)

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РЕЗЮМЕ

Основным преимуществом постоянных линий клеток по сравнению с первичными является возможность наработки стабильного материала, пригодного для продолжительного использования в научно-исследовательских и практических целях. Поэтому важное прикладное значение имеет получение новых перевиваемых культур клеток из разнообразных тканей животных. В статье отражены результаты изучения биологических, цитоморфологических и кариологических особенностей постоянных линий клеток, полученных из органов домашней свиньи (*Sus scrofa* L.), подтверждена чувствительность данных культур к различным вирусам животных, а также описан процесс получения новой диплоидной культуры клеток из селезенки свиньи (SSs – *Splen Sus scrofa*). При анализе полученных данных пришли к выводу, что полноценной иммортализации подвергаются эпителиальные клетки, полученные из почек свиньи после трипсинизации. Все постоянные линии клеток свиного происхождения имеют схожую морфологию с преобладанием эпителиоподобных форм. Некоторые из них – СПЭВ, A_4C_2, RSK – имеют тенденцию переживания сферической формы в суспензии. Такие клеточные линии, как ПСГК-30 и ППЭС,

формируют частичный полислой либо для них характерно значительное уплотнение монослоя с образованием псевдосинцития. Только одна псевдодиплоидная клеточная культура СПЭВ имеет тенденцию к росту в суспензии, она также растет во вращающихся культуральных флаконах. Кариологические трансформации у разных культур стабилизировались на определенном уровне. Спонтанное увеличение количества хромосом в основной популяции постоянных линий клеток в сторону триплоидии привело к стабилизации культуральных свойств и увеличению пролиферации. Наивысший модальный класс – 64 хромосомы – имеет культура ПСГК-30. Околодиплоидные культуры (A_4C_2, RSK) характеризуются стабильными ростовыми параметрами и показывают сходство с культурой СПЭВ в отношении формирования переживающих сферических клеток в среде, качества монослоя и морфологии клеток. Наиболее пластичной клеточной линией оказалась РК-15, которая в разных условиях культивирования имеет отличительный кариотип при сохранении остальных культуральных свойств. В условиях лаборатории ФГБУ «ВНИИЗЖ» в результате длительного инкубирования, субкультивирования (свыше 80 пассажей) и отбора была получена новая постоянная диплоидная культура клеток SSs, которая при проведении дальнейших пассажей может остаться диплоидной или спонтанно стать гетероплоидной – иммортализованной. Велика вероятность того, что впоследствии гиперплоидность клеток спровоцирует увеличение теломеразной активности, что, в свою очередь, стабилизирует иммортализацию и приведет к увеличению пролиферативной активности. До настоящего времени жизнеспособность клеток поддерживается путем регулярных пересевов (коэффициент пересева – 1:2–1:3), осуществляемых 1–2 раза в неделю.

Ключевые слова: постоянная линия клеток, перевиваемая линия клеток, гибридома, диплоидность, гетероплоидность, модальный класс клеток, пролиферативная активность

Благодарность: Исследование проведено за счет средств ФГБУ «ВНИИЗЖ» в рамках научно-исследовательских работ по теме «Ветеринарное благополучие».

Для цитирования: Манин Б. Л., Трофимова Е. А., Гаврилова В. Л., Пузанкова О. С. Биологическая, цитоморфологическая и кариологическая гетерогенность постоянных линий клеток, полученных из органов домашней свиньи (*Sus scrofa* L.). *Ветеринария сегодня*. 2023; 12 (1): 13–22. DOI: 10.29326/2304-196X-2023-12-1-13-22.

Конфликт интересов: Авторы заявляют об отсутствии конфликта интересов.

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INTRODUCTION

Transformed cell lines (TCLs) including those originating from organs of domestic pig (*Sus scrofa* L.) are widely used in veterinary virology [1]. The cell lines demonstrating intensive proliferation were mainly derived from porcine kidney [2–9]. Attempted development of cell cultures easily propagating in matrix from the thyroid gland [10, 11], testicles [11, 12], intestines [10], spleen [13], synovial membrane [14] and other pig organs as well as attempted development of continuous macrophage/monocyte cell lines [15–23] were not successful due to their low proliferative potential with a split ratio of 1:2–1:3. Therefore, development of TCLs having high proliferative activity and applied significance is of current importance.

Normal karyotype of domestic pig well studied in veterinary medicine was used as a reference for karyological examinations [24, 25]. In contrast to molecular genetic analysis [26], karyological analysis allows identification of qualitative and quantitative changes in karyotypes of main porcine TCLs populations and comparison of their biological and cultural properties [27, 28].

The mains advantage of continuous cell cultures is their homogeneity and relevant stability whereas susceptibility of primary cell cultures to various viruses depends on individual features of the animal. Therefore, development of new continuous cell cultures from various animal tissues is an important task. Porcine spleen (*Spleen Sus scrofa* – SSs) is one of such tissues and development of diploid and continuous cell cultures from porcine spleen is of great practical importance.

A new continuous porcine spleen cell culture is developed as a result of long-term incubation, subcultivation and thorough selection; it has undergone more than 80 passages for two years. The diploid cell viability is maintained by regular re-seeding (split ratio– 1:2–1:3) 1–2 times a week.

The study was aimed at biological, cytomorphological and karyological examination of transformed cell lines derived from domestic pig organs (*Sus scrofa* L.), as well as description of the procedure for new diploid cell culture from swine spleen (SSs) development.

MATERIALS AND METHODS

The cell lines were phenotyped using Olympus CKX41 phase-contrast microscope (Japan) and ML-2B luminescent microscope (Russia).

Karyological method for metaphase plate preparation proposed by P. S. Moorhead et al. [24, 28, 29] was used for cell culture identification.

The cells were cultured in conventional media: MEM, DMEM, DMEM/F-12 supplemented with 10% bovine serum.

RESULTS AND DISCUSSION

The following porcine cell lines and sublines are used in Russian veterinary practice: IB-RS-2, SPEV, A_4C_2, A_4C_2/9k, A_4C, PK-15, SK-6, PPES, PPK, PSGK-30, RSK,

Table
Main characteristics of transformed cell lines of porcine origin

No.	Transformed cell lines	Split ratio	Karyology, modal class	Cell monolayer morphology
1	IB-RS-2 (porcine kidney)	1:3; 1:4	36	polygonal, epithelium-like
2	SPEV (porcine kidney)	1:4; 1:6	38	polygonal, epithelium-like, spherical
3	A_4C_2 (SPEV and porcine splenocyte hybrid)	1:3; 1:4	39	polygonal, epithelium-like, spherical
4	RSK (rabbit skin)	1:4; 1:6	40	polygonal, epithelium-like, spherical
5	PPES (porcine kidney)	1:4; 1:6	51	polygonal, epithelium-like
6	PK-15 (porcine kidney)	1:4; 1:6	53	polygonal, epithelium-like
7	PSGK-30 (Siberian ibex kidney)	1:4; 1:18	64	polygonal, epithelium-like
8	ST (swine testicles)	1:2	38	polygonal, epithelium-like
9	SSs (swine spleen)	1:2	38	polygonal, epithelium-like

KST [1, 2, 4, 5, 9, 25, 30–33] derived from kidneys, ST derived from testicles [11, 12], SSs derived from spleen [13].

There are 9 types of transformed porcine cell lines in the FGBI "ARRIAH" Collection (Table).

Examinations of lactate dehydrogenase isoenzyme spectra allowed us to identify RSK (rabbit skin) and PSGK-30 (Siberian ibex kidney) cell lines kept in the FGBI "ARRIAH" Collection as porcine ones. Data on the species identification of given cell cultures were supported by results of examination of their karyotypes, morphology and culture properties.

Marker medium-size metacentric chromosomes containing centromeres that were not stained during routine preparation procedure were used as a reference for confirming the cell culture species identification results (Fig. 1). The metacentric/acrocentric chromosome ratio in most cultures of porcine origin is about 2.2 (±5%): there are 26 metacentrics and 12 acrocentrics in primary porcine kidney cell line.

Description of transformed IB-RS-2 cell line. IB-RS-2 cell line is one of the "oldest" ones developed by M. P. de Castro from porcine kidneys in Sao Paulo, Brazil, in 1962.

The monolayer consists of polygonal epithelium-like cells. The overgrown monolayer forms a syncytium (Fig. 2). IB-RS-2 modal class of 36 chromosomes, 49% of populations (Fig. 3) is the lowest among all known porcine cell cultures (Table). This TCL is susceptible to foot-and-mouth disease virus (FMDV), Teschen disease virus, classical swine fever virus (CSFV), African swine fever virus (ASFV), swine vesicular disease virus (SVDV), vesicular exanthema of swine virus and other viruses.

It should be noted that this TCL has moderate proliferative potential (split ratio: 1:3; 1:4). Pseudosyncytium forms

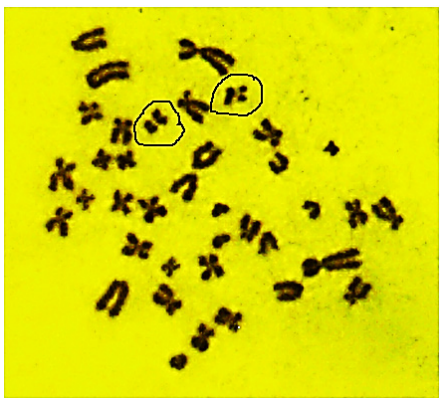

Fig. 1. Porcine diploid chromosome number with two markers and 26 metacentric and 12 acrocentric chromosome ratio

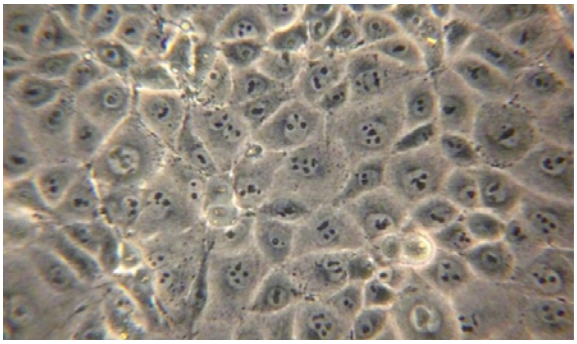

Fig. 2. IB-RS-2 cell line morphology, 40× lens

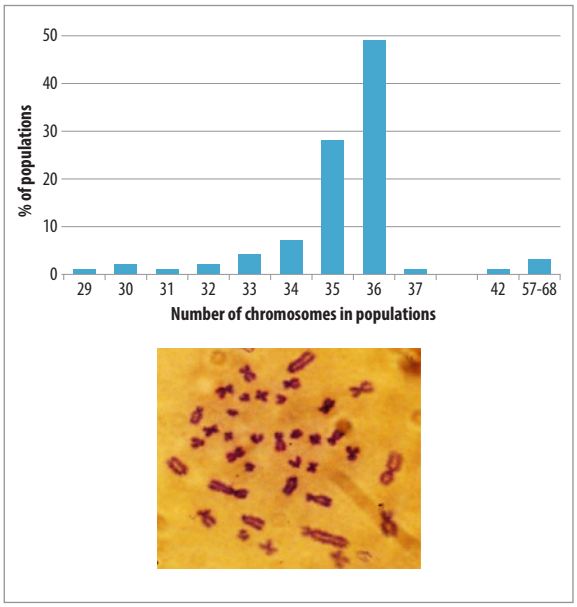

Fig. 3. Karyogram and metaphase plate of IB-RS-2 cell line, 36 chromosomes (26 metacentric and 10 acrocentric chromosomes)

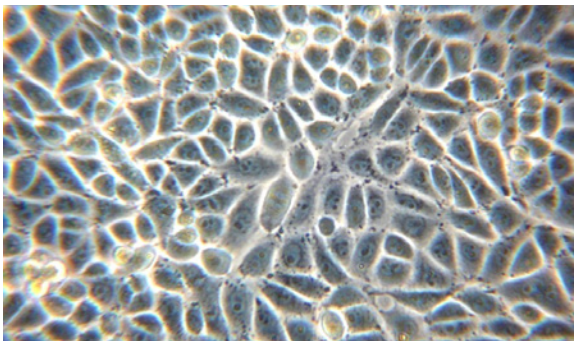

Fig. 4. SPEV cell line morphology, 40× lens

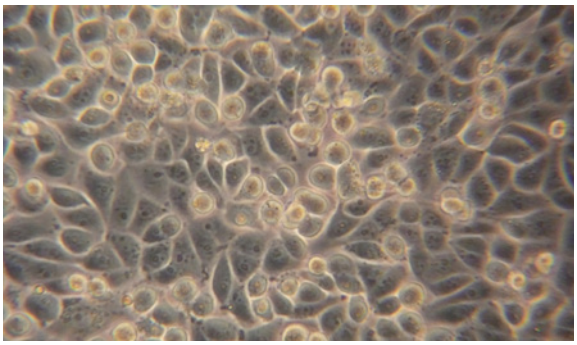

Fig. 6. A_4C_2 cell line morphology, 40× lens

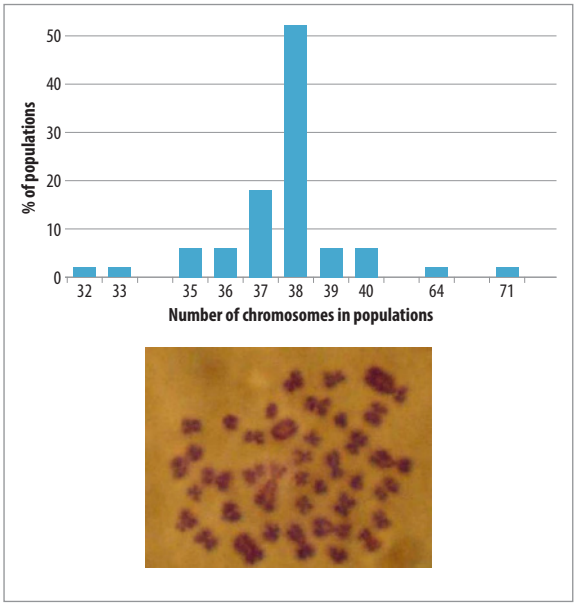

Fig. 5. Karyogram and metaphase plate of SPEV cell line, 38 chromosomes

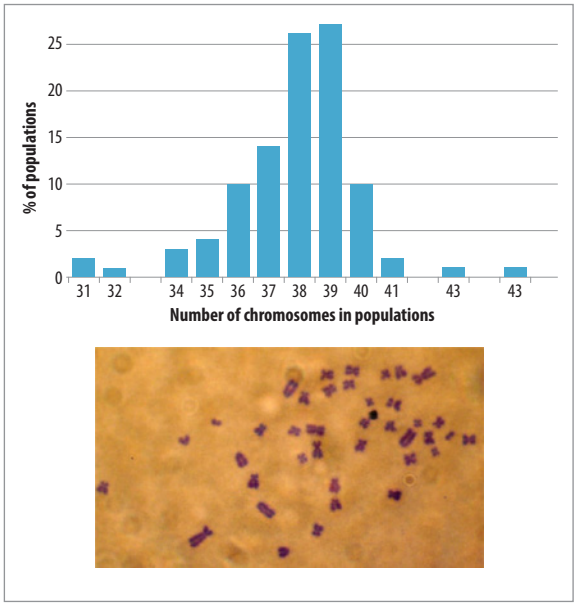

Fig. 7. Karyogram and metaphase plate of A_4C_2 cell line, 39 chromosomes

from stationary-phase monolayer, aggregates can be formed after trypsinization (culture re-seeding), with subsequent formation of colonies during adhesion. The stationary-phase monolayer desintegration is represented by degeneration.

Description of transformed SPEV cell line. SPEV cell line was developed by K. S. Kulikova et al. at the Moscow Research Institute of Virus Preparations in 1959. The cells have a polygonal and epithelial-like shape with rounded nuclei and 2–3 nucleoli (Fig. 4). The modal class – 38 chromosomes, 52% of populations (Fig. 5). Split ratio – 1:4–1:6. This is the only cell line of porcine origin that adapts to growth in suspension and easily cultivated in roller flasks. The cell line is susceptible to FMDV, rinderpest virus (RPV), CSFV, ASFV, transmissible gastroenteritis virus (TGEV), Aujeszky's disease virus (ADV) and other mammal disease agents. Contrary to IB-RS-2, stationary-phase SPEV cell line does not form pseudosyncytium, some cells become suspended and can exist in the suspension for a long time and divide if the limiting proliferation factors are not exhausted.

Description of transformed A_4C_2 cell line. Hybrid A_4C_2 cell line developed through co-cultivation of porcine splenocytes with SPEV cell line by L. P. Dyakonov et al. in the FSC VIEV (Moscow) in 1995 is one of the unique transformed cell lines. Monolayer cells similar to SPEV cell line are of polygonal and epithelium-like shape with rounded nuclei and 2–5 nucleoli (Fig. 6). Modal class – 39 chromosomes (Fig. 7). Its yield during cultivation in rotating flasks is lower than that one of SPEV cell line but similar tendency to cell detachment from the monolayer is observed. A_4C_2 cell line similar to SPEV cell line is susceptible to FMDV, RPV, CSFV, ASFV, TGEV, ADV and other mammal disease agents.

Cells of SPEV line were found to be predominant in hybrid A_4C_2 culture based on morphological and cultural characteristics. The karyotype was transformed, the modal class increased by one chromosome. The long-term co-cultivation with splenocytes appeared to result in proliferation decrease and karyotype transformation. At the same time, the susceptibility to viruses has not changed. Since proliferation intensity was lower than that one of SPEV the split ratio was 1:3; 1:4.

Description of transformed RSK cell line. The cell lines obtained from other institutions are subjected to tests for their morphological and karyological identification at the FGBI "ARRIAH". Thus, RSK cell culture (rabbit skin) obtained from the FSC VIEV (Moscow) was found to be non-susceptible to dermatotropic poxviruses and lumpy skin disease virus. Morphological and karyological examination of the cell line showed its significant similarity to SPEV cell line.

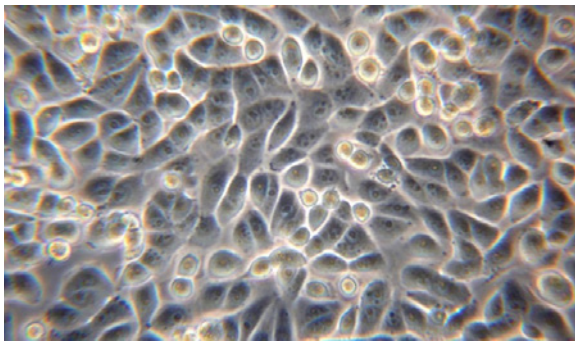

Fig. 8. RSK cell line morphology, 40× lens

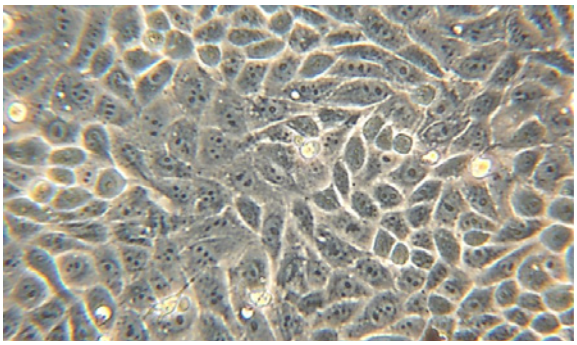

Fig. 10. PPES cell line morphology, 40× lens

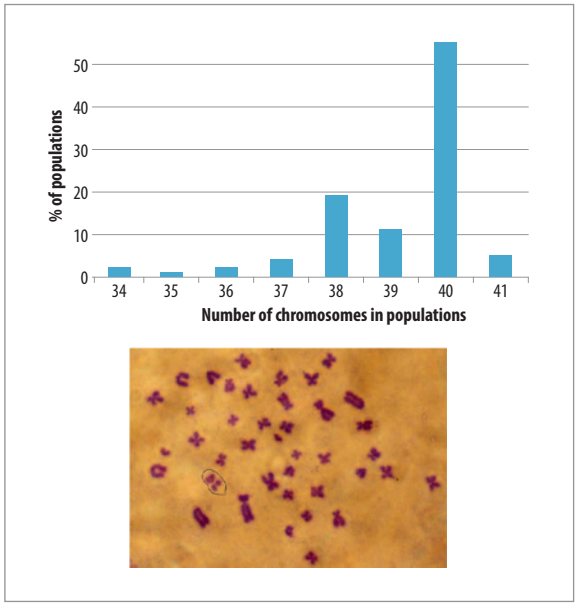

Fig. 9. Karyogram and metaphase plate of RSK cell line, 40 chromosomes

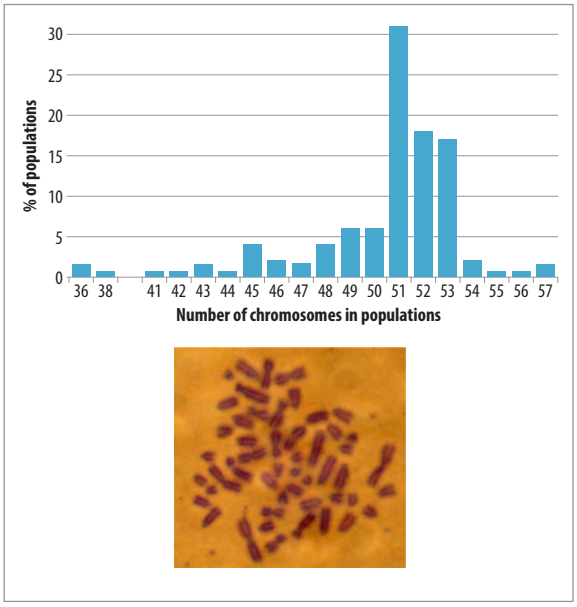

Fig. 11. Karyogram and metaphase plate of PPES cell line, 51 chromosomes

RSK monolayer consists of epithelium-like and rounded cells (Fig. 8). Modal class – 40 chromosomes, 55% of populations (Fig. 9). Chromosome morphology and some markers (medium-size submetacentric with non-stained metacentric bundle) indicate that given transformed cell line is of porcine origin. Split ratio is 1:4; 1:6. The cell line is also suitable for roller cultivation. It is found to be susceptible to TGEV, CSFV, as well as infectious bovine rhinotracheitis, equine rhinopneumonitis, porcine rotavirus infection agents.

We suppose that the rabbit skin cell line was contaminated by SPEV cells, which displaced RSK cells after long-term cultivation. At the same time, its karyotype transformed towards stable hyperploidy.

Description of transformed PPES cell line. PPES (continuous porcine embryo kidney) cell line developed by S. Kh. Khaertynov and G. N. Romanovich at the FSBSI "FCTRBS-ARRVI" (Kazan, Russia) in 1975 is one of the domestic promising and fast-growing transformed cell lines. The monolayer consists of polygonal epithelium-like cells and formed colonies of polylayer (Fig. 10). Modal class – 51 chromosomes, 31% of populations (Fig. 11).

This cell line is characterized by hyperdiploidy. The same tendency is observed in other cell lines of porcine origin. Increase in chromosome number in karyotype have had no effect on the proliferation intensity. On the contrary, the transformed PPES cell line split ratio is 1:4; 1:6. In particular, it is capable of growing in rotating (roller) vessels that is not typical for hyperdiploid cultures. PPES cell line is also characterized by absence of significant mycoplasma and virus contamination and therefore it is capable of long-term continuous passaging. Despite of its good culture and cytomorphological properties, the growth rate of porcine viruses, such as CSFV, TGEV, enterovirus, in this cell culture is low.

Continuous cell lines, PPK and PPK-66b (Kazan line), were prepared from PPES cell line. These cell lines have become more susceptible to porcine disease agents, for example, to porcine parvovirus, after long-term passaging in different media and sera but the cultures are found to be chronically contaminated with mycoplasmas and therefore have limited potential for continuous passaging without treatment with "strong" antibiotics (up to 10 passages). Continuous PPK and PPK-66b (Kazan line) are hyperploid and have modal class of 57 chromosomes in the karyotype. They are not capable of roller cultivation.

Description of transformed PK-15 cell line (FGBI "ARRIAH"). PK-15 cell culture is hyperdiploid. It was developed in the University of California, San-Diego (USA) in 1968. It was obtained by the FGBI "ARRIAH" from Friedrich Loeffler Institute (Germany) in 1986.

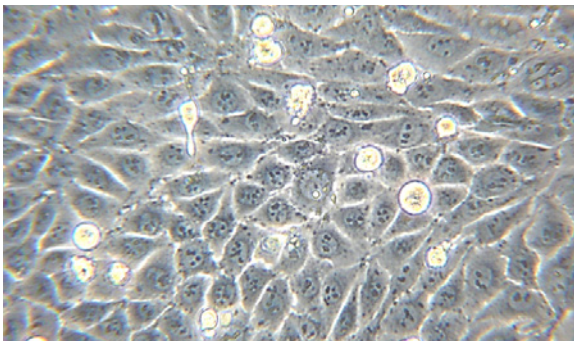

Fig. 12. PK-15 cell line (FGBI "ARRIAH") morphology, 40× lens

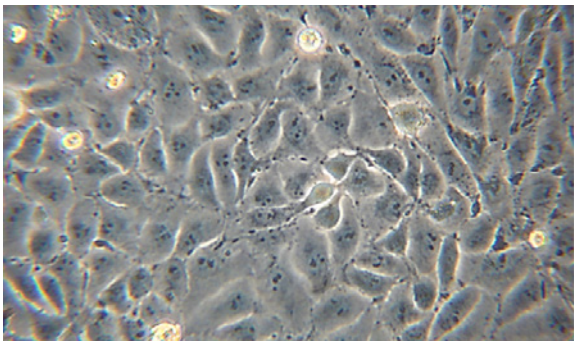

Fig. 14. PK-15 cell line (ATCC) morphology, 40× lens

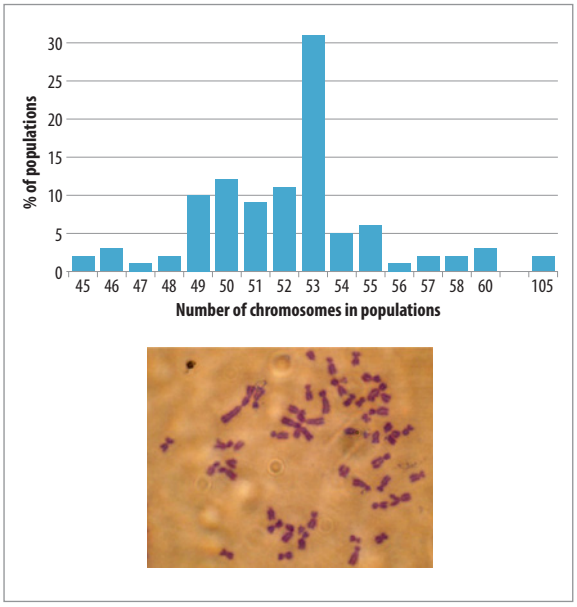

Fig. 13. Karyogram and metaphase plate of PK-15 cell line (FGBI "ARRIAH"), 53 chromosomes

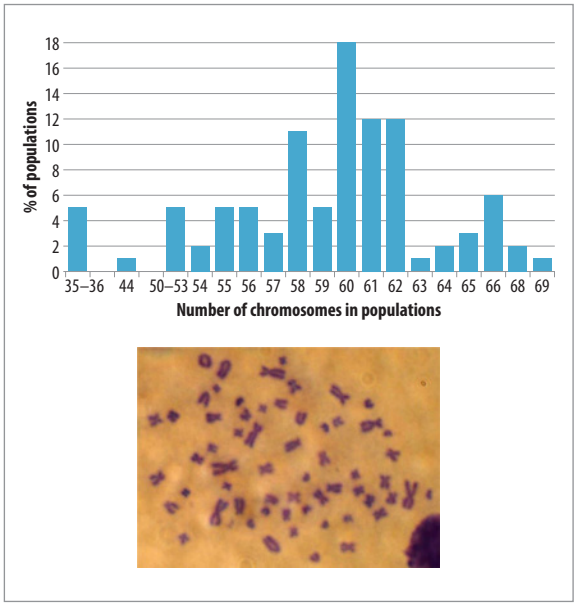

Fig. 15. Karyogram and metaphase plate of PK-15 cell line (ATCC), 60 chromosomes

The cell culture consists of epithelium-like cells, 10–14 mm in size (Fig. 12). Its modal class is 53 chromosomes (Fig. 13). Proliferation intensity is consistent with split ratio of 1:4 and 1:6. This transformed cell line is able to form a complete monolayer in rotating flasks. Its yield reaches 300 mln cells per roller flask (800 cm^2) in 3–4 days. Multinucleated cells (1–2%) form in the overgrown PK-15 monolayers in culture flasks.

The cell line is susceptible to ASFV, CSFV, ADV, vesicular stomatitis virus (VSV), Coxsackievirus, vaccinia virus (VACV), porcine circovirus (PCV), reovirus serotype 2 and 3, adenovirus serotype 4 and 5 and other mammal disease agents.

Description of transformed PK-15 cell culture (ATCC, American Type Culture Collection). PK-15 cell trophovariants obtained from Hungary and the ATCC have a modal class of 60 chromosomes (Fig. 14). Cell and monolayer morphology is identical to those of PK-15 culture described above (Fig. 15). Cell yield and proliferation intensity are also similar. Differences in the karyotype can be accounted for different cultivation conditions in different laboratories. In European laboratories full synthetic media are predominantly used for cultivation, whereas protein hydrolysates are often used at the FGBI "ARRIAH".

The cell line is susceptible to ASFV, CSFV, ADV, VSV, Coxsackievirus, VACV, PCV, reovirus serotype 2 and 3, adenovirus serotype 4 and 5, and other mammal disease agents.

Description of transformed PSGK-30 cell line. Continuous PSGK cell line (continuous Siberian ibex kidney cell line) was developed by I. G. Kekukh, L. P. Kiryukhina, Z. M. Lukyanova in the Research Institute of Agriculture (RIAC) of the MOA of USSR in 1976. There are the following trophovariants and sublines of the said cells: PSGK, PSGK-30, PSGK-60, PSGK-c60 and PSGK-c85.

Several researchers found that this cell culture was contaminated with the cells of porcine origin (V. G. Kostyuchenko, et al., 1985; N. Yu. Smyslova et al., 1996). Currently, PSGK-30 cell line is a highly transformed porcine culture that has formed as a result of contamination of primary Siberian ibex kidney cell culture with more viable transformed SPEV, PPK or PK-15 cell cultures.

PSGK-30 cell culture is one of the most active porcine cell cultures having high proliferation index up to 3.0. The split ratio can reach 1:20. High proliferation potential is achieved by optimization of the nutrient medium containing lactalbumin hydrolysate at concentration of 0.1%. The cell culture monolayer consists of epithelium-like cells (Fig. 16). The karyotype modal class is

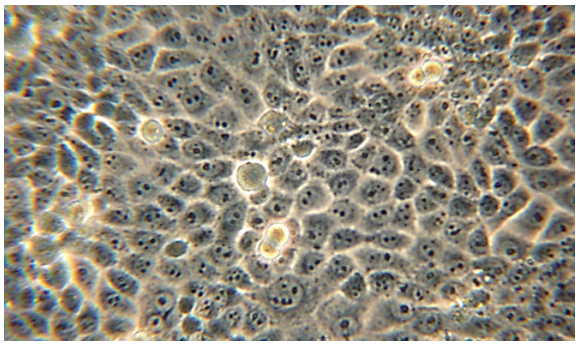

Fig. 16. PSGK-30 cell line morphology, 40× lens

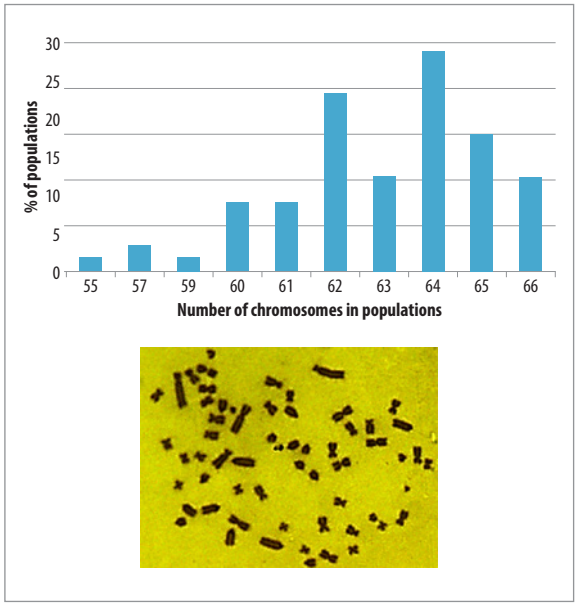

Fig. 17. Karyogram and metaphase plate of PSGK-30 cell line, 64 chromosomes

64 chromosomes, the highest one among the cultures of porcine origin (Fig. 17). The stationary-phase TCL monolayer is so dense that the cells become compacted with formation of epithelium-like polylayer in some layer sections. Conglomerates of the cells often form after trypsinization for reseeding that form growth colonies when sedimented and adhered. The cell yield from one cultivation flask is not higher than 120 mln cells. Transformed PSGK-30 is the main substrate for cultivation of master seed FMDV of all strains used for the vaccine production.

Description of transformed ST cell line. Cell lines derived from other pig organs, porcine testicles and spleen, form a specific group of TCLs. ST (swine testicles) cell line has low proliferative activity. At split ratio of 1:2 nonconfluent monolayer forms for 7–10 days. The cell cycle takes several days. The monolayer consists of large epithelium-like and spindle-shaped cells. Extracellular matrix develops in stationary-phase monolayer (Fig. 18).

Weak proliferative activity makes difficult the collection of the dividing cells used for karyological preparations. But even though its karyotype can be determined as diploid (Fig. 19).

ST cell culture has diploid karyotype with about 38 chromosomes. But it technically impossible to determine its modal class due to absence of sufficient number

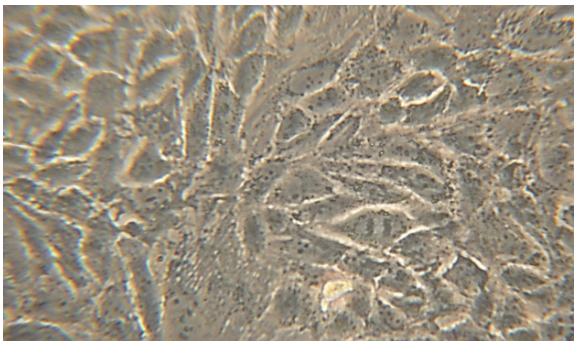

Fig. 18. ST cell line morphology, 40× lens

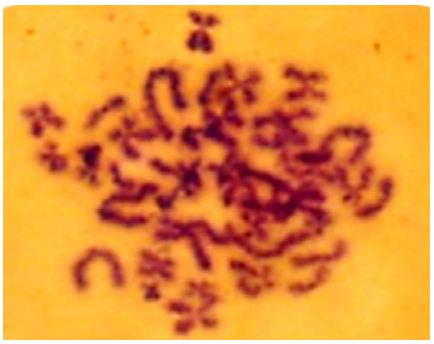

Fig. 19. Metaphase plate of ST cell line, about 38 chromosomes

of metaphase plates when standard karyotyping method is used.

Transformed ST cell line is susceptible to many viruses affecting pigs (*Sus scrofa*), but it is not used for production of diagnostica and specific vaccines due to its low proliferative activity (1:2), the cell monolayer forms within 1–1.5 weeks. Transformed ST cell line yielding potential has not been studied, and this is indicated in the data sheet for this cell line.

Development of diploid transformed SSs cell line. During standard operations for preparation of primary cells from animal organs at the Cell Cultivation Unit of the FGBI "ARRIAH", subcultivation of trypsinized piglet spleen cells using a semi-synthetic nutrient medium + DMEM/F-12, at ratio of 1:2–1:3 supplemented with 10% bovine serum treated with lanthanoides was attempted. This TCL was preliminary named as SSs (*Spleen Sus scrofa* – swine spleen).

At the first passages, the subculture consisted of mixed cell population with a predominating epithelium-like cells, which formed colonies evenly distributed over the entire culture surface of the flasks, then the colonies merged into a confluent cell monolayer. After trypsinization, the cells were large up to 20 μm, characterized by polymorphism and incomplete monolayer confluence (Fig. 20*a*).

As the diploid cells of the swine spleen were further passaged, their proliferative activity increased, the culture became morphologically more homogeneous, consisting of polygonal-shaped epithelium-like cells with clear, well-defined borders and rounded nuclei (with 1–3 nucleoli) and clear sometimes vacuolated cytoplasm.

Atypical dynamics was observed during subcultivation of the swine spleen cell line. The monolayer formed for 10 days by the 40th passage (in fact, by the 40th reseeding).

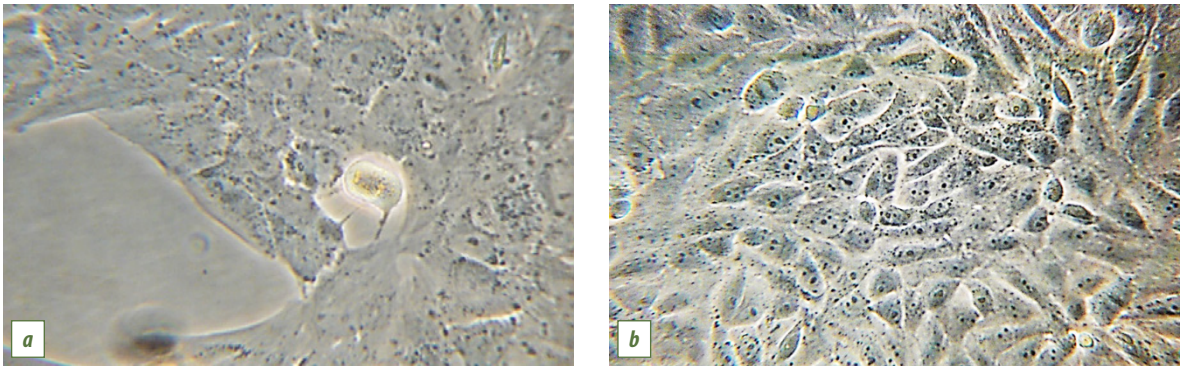

Fig. 20. SSs cell line morphology: a) at 6th passage; b) at 74th passage (40× lens)

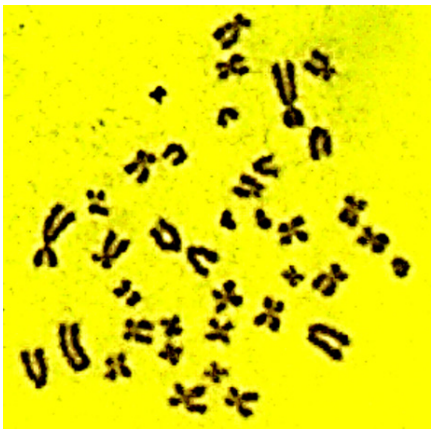

Fig. 21. Diploid metaphase plate of continuous SSs cell line, 74th passage, 38 chromosomes

By this passage, the size of the cells decreased, the density increased, but the number of mitoses remained at the same level. After reseeding (the cells were harvested from the substrate using trypsin-versene solution), sedimentation, adhesion and flattening of the cells took place within 24–36 hours. The medium was significantly acidified after 96 hours, but the monolayer did not form. Almost confluent monolayer formed when the medium was changed.

Transformed SSs cell line maintains reproduction of ASFV, CSFV, TGEV and porcine reproductive and respiratory syndrome virus.

The next step was to intensify proliferation through selection of nutrient media and cultivation conditions as well as to determine cell susceptibility to other animal viruses. SSs cell culture was adapted to minimum essential medium (MEM).

By 74th passage the cell became more morphologically homogenous with predominant epithelium-like cells. Cells in confluent monolayer were 15 μm in size (Fig. 20*b*). Proliferation intensity remained low: split ratio of 1:2; 1:3 after 4–6 days. Developed transformed SSs cell line was susceptible to CSFV, ASFV, ADV, TGEV and other porcine disease agents. The transformed SSs cell line was used for research only due to its low proliferative activity.

Karyological examinations showed that transformed SSs cell population contained mainly diploid cells (Fig. 21).

In our opinion, only cells of stromal origin in the transformed cell line population that *in vivo* did not demonstrate intensive proliferation were transformed towards immortalization.

CONCLUSION

Analysis of cyto-morphological and biological properties of transformed cell lines of porcine origin allows us to conclude that epithelial cells derived from trypcinized porcine kidney can be completely immortalized. All continuous porcine cell lines have similar morphology with predominate epithelium-like forms. Some of them, SPEV, A_4C_2, RSK, tend to adopt a spherical form in suspension. Some TLCs such as PSGK-30 and PPES, form a partial polylayer and characterized by significant monolayer compaction with pseudosyncitium formation. SPEV is the only one pseudodiploid cell culture that tends to grow in suspension, it also grows in rotating culture flasks.

Karyological transformations in different cultures stabilized at certain level. Spontaneous increase in chromosome numbers in main populations of transformed cell lines towards triploidy resulted in stabilization of culture properties and increased proliferation.

Near-diploid cultures (A_4C_2, RSK) also demonstrated stable growth parameters and trend for spherical cell formation in the medium as well as were similar to SPEV cell culture in monolayer quality and cell morphology.

Cell cultures derived from other pig organs: porcine testicles and spleen (ST, SSs) form a specific group. We suppose that these TCLs have originated from stromal cells and are characterized by low proliferative activity and diploid cell populations. Such TCLs are largely of interest to researchers.

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Received 18.01.2023
Revised 01.02.2023
Accepted 09.02.2023

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