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Comparison of gene expression of metallothioneins, ubiquitin and p53 in fibroblasts from lung and skin of rats of different age

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We studied gene expression of five metallothioneins (MT 1-5), ubiquitin and protein p53 and their products in fibroblasts culture of the skin and lungs of white rats of different ages (2 weeks, 1, 3, and 24 months) and determined its (metallothionein 1-5 types, ubiquitin, p53) product quantity. All these proteins are protective ones, but perform their functions by using different mechanisms. Metallothionein bind, transport and excrete ions of bivalent metals, ubiquitin controls the cleavage of the defective and short-lived proteins in the proteasome, protein p53 controls apoptosis, thus ensuring the genome stability. The similarity of age dynamics of gene expression of ubiquitin and MT of cells of both sources has been shown – maximum at 3 months. Expression of p53 gene has a difference: both in the skin and lungs expression increases up to 24 months. Product quantity of p53 has a minimum in the skin at 3 months and remains constant; in the lungs, this value has a maximum at 1 month.

Keywords: culture of fibroblasts; ontogeny; metallothioneins; ubiquitin; p53

Сравнение экспрессии генов металлотионеинов, убиквитина и p53 в фибробластах легких и кожи крыс разного возраста

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Проведено исследование экспрессии генов пяти металлотионеинов (МТ 1-5), убиквитина и белка p53 в культуре фибробластов кожи и легких белых крыс разного возраста (2 недели, 1, 3 и 24 месяца), а также определено количество их продуктов (металлотионеинов 1–5 типов, убиквитина, белка p53). Все эти белки являются защитными, но выполняют свои функции с использованием разных механизмов. Металлотионеины связывают, транспортируют и выводят из организма ионы двухвалентных металлов, убиквитин контролирует расщепление в протеасомах дефектных и короткоживущих белков, белок p53 контролирует механизм апоптоза, обеспечивая таким образом стабильность генома. В связи с этим задачей настоящей работы было сравнение экспрессии указанных генов и количества их белковых продуктов в фибробластах соединительной ткани кожи и легких для выявления роли генетических факторов в ходе их онтогенеза. Показано сходство возрастной динамики экспрессии генов МТ и убиквитина из клеток обоих источников – наличие максимума в 3 месяца. Возрастные изменения экспрессии генов всех пяти МТ характеризуются как в коже, так и в легких подъемом в первой половине онтогенеза. Для кожи характерен четкий максимум экспрессии генов МТ в 3 месяца, для легких этот максимум менее четок. К 24-му месяцу экспрессия всех генов МТ в фибробластах как кожи, так и легких, резко падает в 2–3 раза по сравнению с максимумом. Экспрессия гена убиквитина, контролирующего расщепление короткоживущих или поврежденных белков, увеличивается в фибробластах легких к 3 месяцам в 1,5 раза, а далее снижается ниже уровня у двухнедельных животных. В коже максимум экспрессии данного гена также приходится на 3 месяца, но снижение не такое существенное. Количество продукта убиквитинового гена в легких возрастает до трехмесячного возраста и далее практически не меняется. В коже наблюдается падение в период от двух недель до одного месяца, подъем к трем месяцам и резкий спад к старости (примерно в 3 раза по сравнению с трехмесячным возрастом). Экспрессия гена p53 имеет иной характер: и в коже, и в легких повышение экспрессии происходит вплоть до 24 месяцев. Количество продукта p53 минимально в коже в 3 месяца и далее не изменяется; в легких эта величина максимальна в один месяц.

Ключевые слова: культура фибробластов; онтогенез; металлотионеины; убиквитин; p53

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Introduction

Fibroblasts in cell culture from lungs and skin of white rats of different age (2 weeks, 1, 3, and 24 months) have been studied. Numerous works in the field of developmental biochemistry are still held mainly at the organ and tissue level without the use of experiments with cell cultures (Muller, 2007). Meanwhile, using for such purposes the culture of cells in a complex picture of the ontogenetic changes helps to isolate the effect of genetic factors. Objects of research are fibroblasts from various sources, having the same origin, but functioning in different organs (Chang, 2002; Al Bahar, 2004; Ring, 2006), they have varying degrees of expression of certain determined genes. Gene expression of several proteins with different mechanism of action and the quantity of their products has been investigated (metallothioneins 1–5 types, ubiquitin, p53). All these proteins are protective ones, but perform their function using different mechanisms.

Metallothionein bind, transport and excrete ions of bivalent metals (Davis, 2000; Pyhteeva, 2009; Thirumoorthy, 2011). Metallothionein expression is driven by a number of physiological factors through several response elements in metallothionein gene promoter. Cellular accumulation of these proteins depends on both gene expression and protein degradation. Both depend largely on the availability of cellular zinc derived from the dietary zinc supply. Metallothioneins could act in a number of biochemical processes. For example, they can take part in trafficking and donation of zinc to appropriate apoproteins, including zinc finger proteins. As a result, metallothioneins may affect a number of cellular processes (gene expression, apoptosis, and proliferation). Metallothioneins have the ability to exchange other metals with zinc in different metalloproteins, and it can explain its role in metal toxicity. Mobilization of zinc and cuprum from metallothioneins by oxidative stress may explain its antioxidant function (Liuzzi, 2001).

Ubiquitin controls the cleavage of the defective and short-lived proteins in the proteasome (Li, 2008). Selective degradation of many short-lived proteins is carried out by the ubiquitin system. Ubiquitin-mediated degradation of regulatory proteins plays important role in the control of numerous processes, including cell cycle, signal transduction transcription regulation, endocytosis. The ubiquitin system is implicated in degradation of abnormal proteins that have defects as a result of gene mutation or some post-translation damage (Barder, 2006; Hoyt, 2006).

The protein p53 controls apoptosis, thus ensuring genome stability (Chumakov, 2007). Signals for metabolic processes diverge from the optimum or for proteins structure damage converge on p53 through its numerous connections with various proteins (Vousden, 2007). Depending on the damage level, the result of p53 activity is either acceleration of DNA repair or stoppage of cell division and apoptosis (Degterev, 2008).

In this regard, the purpose of this study was the comparison of the expression of these genes and their protein products in the connective tissue of skin and lungs to determine the role of genetic factors in the course of their ontogenesis.

Materials and methods

The experiments were conducted in accordance with the international instruments on bioethics (European Convention "About protection of vertebrate animals used for experimental and other scientific purposes", the Law of Ukraine "On protection of animals from cruelty"). Donors of skin and lung fibroblasts were purebred white rats of 4 age groups (2 weeks, 1 month, 3 month and 24 month).

Cell isolation. Lungs and skin milled in DMEM medium, containing 1% Trypsin. After 30-minute incubation at 37 °C cells were harvested and seeded in vented culture flasks in DMEM medium, containing 10% FBS, and culturing was carried out.

Cell cultivation. Cells were cultured at 37°C and 95% humidity in the presence of 5% CO_2 (Nuair 4500, USA). Cell attachment and density of the cell culture was monitored in transmitted light of the inverted microscope Carl Zeiss Telaval. We used fibroblasts of the 3rd passage.

Analysis of gene expression was performed on Arrayit DNA-microarray of production Arrayit (Arrayit, USA).

Total RNA was isolated from the cells with spin-column set of RNeasy Mini Kit (Qiagen) according to the original manufacturer's manual. cDNA synthesis by reverse transcription was carried using QIAGEN OneStep RT-PCR Kit (Qiagen, USA). We used gene-specific primers and Cy3-labeled nucleotides manufactured by Arrayit and Life Technologies (USA), respectively. Hybridization was carried out using individual chambers for hybridization SecureSeal (Invitrogen, USA), in separate wells that contribute labeled cDNA from the cells of animals of different age.

Final amount of protein product made was determined using microarray ELISA-kits and reagents Antibody Array Assay Kit (KAS20, Full Moon BioSystems, Inc., USA).

The chips were scanned with the confocal fluorescence scanner Affymetrix 428 using the Jaguar software. The obtained results are expressed as fluorescence units – rFLU per 1 cell.

The results were statistically processed using the Mann – Whitney test. Results were expressed as M ± SD, where M – arithmetic mean, SD – standard deviation. Results with $P < 0.05$ were considered reliable.

Results and discussion

One of the important mechanisms of adaptation to high doses of heavy metals and of metals of variable valence is to increase the gene expression of metallothioneins (MT), low molecular weight proteins, containing in its composition high percentage of cysteine clusters (Pykhteeva, 2009; Thirumoorthy, 2011). Due to this MT is capable of binding divalent metals with high affinity. Now there are found more than 10 isoforms of these proteins in different living facilities (Davis and Causins, 2000). MT bind both essential metals (copper, zinc, selenium, ensuring their transport to the sites of utilization or excretion when excess flow) and metals-toxicants (cadmium, mercury, lead, etc.). MT are found in almost all organs of mammals. The destruction of the complex of MT with metals, at least for some of the isoforms, occurs in the lysosomes of the kidneys, and then released in the form of ionic metals excreted in the urine.

Age-related changes of gene expression of all five MT are characterized by a rise in the first half of ontogeny both in skin and lungs (Table 1). For skin characterized by the clear maximum of MT gene expression in 3 months, for light this maximum is less clear. For 24 months, the expression of all MT genes in fibroblasts of skin and lungs sharply decreases 2–3 times compared to the maximum.

Almost all age groups have the higher level of protein-MT in skin compared to the lungs, and sharp decline in the amount of product is observed by 24 months (Table 2). Changes in ontogenesis are less expressed and have the opposite direction to MT 1-3 or reaching the maximum at 3 months for MT 4 and 5 in lung tissue.

Gene expression of ubiquitin (Table 3) that controls the splitting of short-lived or damaged proteins (Li, 2008), increases in lung fibroblast at 3 months 1.5 times, and further it decreases below the level in 2 week animals. In skin high expression of this gene is also observed for 3 months, but the decline is not so significant.

Table 1

Gene expression of metallothionein in cultured fibroblasts of skin and lungs in rats of different age (rFLU per 1 cell)

Gen (Protein)	Tissue	Expression			
		0.5 month	1 month	3 month	24 month
MTL1 (MT1)	skin	33.0 ± 2.0	116.0 ± 7.0^*	144.1 ± 8.0^*	51.1 ± 3.1^*
	lung	59.0 ± 1.0	74.0 ± 1.0^*	118.3 ± 2.0^*	48.2 ± 1.0^*
MTL2 (MT2)	skin	2.0 ± 0.0	59.0 ± 4.0^*	56.1 ± 4.0^	15.0 ± 1.0^*
	lung	22.0 ± 2.0	41.1 ± 4.0^*	49.0 ± 5.0^	17.1 ± 2.0^*
MTL3 (MT3)	skin	8.0 ± 1.0	52.0 ± 4.0^*	56.1 ± 4.0^	16.0 ± 1.0^*
	lung	30.0 ± 1.0	37.1 ± 1.0^*	49.1 ± 1.0^*	18.0 ± 1.0^*
MTL4 (MT4)	skin	7.0 ± 1.0	48.3 ± 3.1^*	44.0 ± 3.0^	20.1 ± 1.1^*
	lung	28.0 ± 2.0	35.0 ± 2.0^*	40.0 ± 3.0^	21.0 ± 1.1^*
MTL5 (MT5)	skin	43.1 ± 3.0	140.1 ± 9.1^*	97.1 ± 6.3^*	52.1 ± 3.0*
	lung	71.1 ± 4.1	88.1 ± 5.2^*	81.1 ± 5.2^	49.1 ± 3.0^*

Note: * – significantly ($P < 0.05$) compared to the previous age; ^ – significantly ($P < 0.05$) relative to 0.5 months.

Table 2

Product quantity of metallothionein in cultured fibroblasts of skin and lungs in rats of different age (rFLU per 1 cell)

Gen (Protein)	Tissue	Product quantity			
		0.5 month	1 month	3 month	24 month
MTL1 (MT1)	skin	822.4 ± 168.3	605.4 ± 124.2	399.5 ± 82.3^	169.1 ± 35.4^*
	lung	140.4 ± 25.2	157.1 ± 27.3	168.1 ± 29.0	228.4 ± 40.1^
MTL2 (MT2)	skin	764.1 ± 185.0	499.4 ± 121.3	261.1 ± 63.0^*	127.6 ± 31.1^*
	lung	84.3 ± 19.2	94.4 ± 21.0	115.0 ± 25.2	133.3 ± 29.2^
MTL3 (MT3)	skin	777.4 ± 167.1	487.3 ± 105.5^*	261.8 ± 55.2^*	128.0 ± 27.0^*
	lung	64.3 ± 6.0	72.6 ± 7.7	106.3 ± 10.5^*	114.7 ± 11.5^
MTL4 (MT4)	skin	774.1 ± 199.4	487.1 ± 125.7	243.2 ± 62.9^*	128.7 ± 33.7^*
	lung	74.0 ± 8.1	83.4 ± 8.2	102.1 ± 10.0^*	97.5 ± 10.2^
MTL5 (MT5)	skin	841.1 ± 198.2	649.8 ± 153.2	325.8 ± 77.0^*	170.0 ± 40.0^*
	lung	152.0 ± 6.0	171.6 ± 7.6^*	209.0 ± 9.9^*	168.8 ± 7.7

Note: see Table 1.

Table 3

Gene expression of ubiquitin and p53 in cultured fibroblasts of skin and lungs in rats of different age (rFLU per 1 cell)

Gen (Protein)	Tissue	Gene expression			
		0.5 month	1 month	3 month	24 month
UBB (ubiquitin)	skin	251.1 ± 12.0	312.0 ± 15.1^*	622.0 ± 31.0^*	444.4 ± 22.2^*
	lung	316.0 ± 3.1	424.3 ± 5.1^*	491.4 ± 5.0^*	218.1 ± 2.2^*
TP53 (p53)	skin	33.0 ± 2.0	41.4 ± 3.0^*	63.3 ± 4.5^*	159.6 ± 10.1^*
	lung	59.1 ± 4.1	62.1 ± 4.0	55.4 ± 3.1	143.0 ± 9.0^*

Note: see Table 1.

The amount of ubiquitin gene product in lungs increases for 3 months age and then remains practically unchanged (Table 4). In skin there is a decline in the period from 2 weeks to 1 month, climbing to 3 months and the sharp decline to old age (approximately 3 times compared with 3-month age). Obviously, similarities of the dynamics of gene expression and their products, for MT and ubiquitin, suggests that at young age the ability of fibroblasts of skin and lungs contributes to successful protection of connective tissue from its proteins damage by different external influences. But with age genetically determined adaptability to damaging factors is reduced, and this may partly explain the accumulation of damaged proteins with age in several age-related pathologies.

Gene TP53, which controls genome stability and triggers apoptosis under the action of genome damaging factors (Chumakov, 2007), is maximally expressed in old age both in lungs and skin (Table 3). Quantity of the product is significantly reduced in skin for 3 months and remains virtually unchanged in the second half of ontogeny (Table 4). In the lung fibroblast protein p53 fluctuates less, its maximum is observed in 1 month.

Table 4

Product quantity of ubiquitin and p53 in cultured fibroblasts of skin and lungs in rats of different age (rFLU per 1 cell)

Gen (Protein)	Tissue	Product quantity			
		0.5 month	1 month	3 month	24 month
UBB (ubiquitin)	skin	1226.6 ± 11.4	969.1 ± 9.0^*	1146.4 ± 10.1^*	399.1 ± 3.6^*
	lung	700.7 ± 38.3	787.4 ± 43.2^*	968.2 ± 53.0^*	964.1 ± 52.1^
TP53 (p53)	skin	822.2 ± 3.1	467.1 ± 1.0^*	273.0 ± 1.0^*	297.1 ± 1.0^*
	lung	186.3 ± 6.0	209.2 ± 7.2^*	161.1 ± 5.0^*	149.0 ± 5.0^*

Note: see Table 1.

Obviously, the mechanism of p53 action is significantly more complicated and it does not fit the pattern similar to that of other protective proteins investigated. The differences between the dynamics of p53 gene and its products both in skin and lungs also reveal more complex picture of changes in ontogenesis, which interpretation needs further research.

Conclusions

We found similarities of the gene expression studied at different age, typical to MT and ubiquitin both in skin and lungs. They consist in achieving the maximum expression within 3 months. Dynamics of p53 protein gene expression and its accumulation in fibroblasts differs significantly in ontogenesis from the dynamics of MT and ubiquitin. Besides, essential distinctions in the gene and the protein p53 in fibroblasts of the skin and lungs were found.

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