ФЕДЕРАЛЬНОЕ ГОСУДАРСТВЕННОЕ БЮДЖЕТНОЕ УЧРЕЖДЕНИЕ НАУКИ
ИНСТИТУТ ЦИТОЛОГИИ РОССИЙСКОЙ АКАДЕМИИ НАУК

Направление подготовки 06.06.01 Биологические науки

Специальность 03.01.03 Молекулярная биология

ПОРТФОЛИО АСПИРАНТА

ПАРФЕНЬЕВА Сергея Евгеньевича

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1. Общие сведения

Лаборатория: *регуляции экспрессии генов (РЭГ)***.**
Тема диссертационной работы: *Регуляция транскрипционной активности онкосупрессора p53 в процессе эпителиально-мезенхимального перехода.*
Научный руководитель: *Барлев Николай Анатольевич, д.б.н., профессор.*
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2. Научные публикации

статьи*:*

Genome-wide 5-hydroxymethylcytosine patterns in human spermatogenesis are associated with semen quality. lga A. Efimova, Anna A. Pendina, Andrei V. Tikhonov, ***Sergey E. Parfenyev****, Irina D. Mekina, Evgeniia M. Komarova, Mariia A. Mazilina, Eugene V. Daev, Olga G. Chiryaeva, Ilona A. Galembo, Mikhail I. Krapivin, Oleg S. Glotov, Irina S. Stepanova, Svetlana A. Shlykova, Igor Yu. Kogan, Alexander M. Gzgzyan, Tatyana V. Kuznetzova and Vladislav S. Baranov. Oncotarget, 2017.*

Catechol-O-methyltransferase Val158Met polymorphism is associated with increased risk of multiple uterine leiomyomas either positive or negative for MED12 exon 2 mutations. Lyailya Kh Dzhemlikhanova, Olga A Efimova, Natalia S Osinovskaya, ***Sergey E Parfenyev****, Dariko A Niauri, Iskender Yu Sultanov, Olga V Malysheva, Anna A Pendina, Natalia Yu Shved, Tatyana E Ivashchenko, Maria Yarmolinskaya, Maka I Kakhiani, Ekaterina A Gorovaya, Antonina N Tkachenko, Vladislav S Baranov. Clinical Pathology, 2017.*

тезисы:

European Human Genetics Conference ESHG 2016. P01.003. 5-methylcytosine and 5-hydroxymethylcytosine patterns in human spermatogenic cells. A. V. Tikhonov1,2, O. A. Efimova1,2, A. A. Pendina1,2, I. D. Fedorova2, ***S. E. Parfenyev****1, A. S. Koltsova1,2, T. V. Kuznetzova1,2, V. S. Baranov1,2;1Saint Petersburg State University, St.Petersburg, Russian Federation, 2D.O.Ott Research Institute of Obstetrics,Gynecology and Reproductology, St.Petersburg, Russian Federation.*

главы в книгах или сборниках:

Immunofluorescence Staining for Cytosine Modifications Like 5-Methylcytosine and Its Oxidative Derivatives and FISH. Anna A. Pendina, Olga A. Efimova, Andrei V. Tikhonov, Olga G. Chiryaeva, Irina D. Fedorova, Alla S. Koltsova, Mikhail I. Krapivin, ***Sergey E. Parfenyev****, Tatyana V. Kuznetzova, and Vladislav S. Baranov. Из книги Fluorescence In Situ Hybridization (FISH). EditorThomas Liehr Institut fur Humangenetik Jena, Germany, 2017.*

3. Участие в научных конференциях, симпозиумах, семинарах, выставках

4. Участие в грантах

Композиция эпигенетических модификаций цитозина ДНК на последовательных этапах дифференцировки сперматогенных клеток человека в норме и при нарушениях сперматогенеза. РФФИ № 16-34-00532. Исполнитель. Закончен.

Специфичность паттернов 5-гидроксиметилцитозина в геноме клеток эмбриональных и экстраэмбриональных тканей человека при нормальном развитии и эмбриональных потерях. РФФИ № 16-04-01438. Исполнитель. Закончен.

Трансформированные и раковые стволовые клетки как мишени для противоопухолевых средств. РНФ № 14-50-00068. Временный исполнитель. Выполняется.

Роль онкогена Mdm2 и его сплайс-изоформ в регуляции метастазирования и инвазивности раковых опухолей человека. РНФ № 14-15-00816. Исполнитель. Выполняется.

Убиквитинлигазы MDM2 и PIRH2 как возможные прогностические маркеры HER2-положительного рака молочной железы: их участие в репарации ДНК и устойчивости к химиотерапии. РНФ № 18-75-10076. Ответственный исполнитель. Выполняется.

5. Научно-педагогическая деятельность

6. Дополнительная информация

7. Сведения об освоении основной образовательной программы подготовки научно-педагогических кадров в аспирантуре

Кандидатские экзамены

№ п.п.	Дисциплина	Дата сдачи	Оценка	Место сдачи
1	История и философия науки		хорошо	СПБГУ
2	Английский язык		хорошо	СПБГУ
3				

Приложение 1.

Научные публикации

Размещаются соответствующие копии документов

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No.51), pp: 88294-88307

Research Paper: Pathology

Genome-wide 5-hydroxymethylcytosine patterns in human spermatogenesis are associated with semen quality

Olga A. Efimova[1,2,*], Anna A. Pendina[1,2,3,*], Andrei V. Tikhonov[1,2,3,*], Sergey E. Parfenyev[2], Irina D. Mekina[1], Evgeniia M. Komarova[1], Mariia A. Mazilina[1,2], Eugene V. Daev[2], Olga G. Chiryaeva[1,4,5], Ilona A. Galembo[3], Mikhail I. Krapivin[2], Oleg S. Glotov[1,2], Irina S. Stepanova[6,7], Svetlana A. Shlykova[8], Igor Yu. Kogan[1], Alexander M. Gzgzyan[1,2], Tatyana V. Kuznetzova[1,2] and Vladislav S. Baranov[1,2]

[1] D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russia

[2] St. Petersburg State University, St. Petersburg, Russia

[3] Center for Medical Genetics, St. Petersburg, Russia

[4] St. Petersburg State Pediatric Medical University, St. Petersburg, Russia

[5] S.M. Kirov Military Medical Academy, St. Petersburg, Russia

[6] Institute of Cytology RAS, St. Petersburg, Russia

[7] Aimed Clinic, St. Petersburg, Russia

[8] AVA-Peter Clinic, St. Petersburg, Russia

[*] These authors have contributed equally to this work

Correspondence to: *Olga A. Efimova,* ***email:*** *efimova_o82@mail.ru*

Keywords: 5-hydroxymethylcytosine, semen quality, sperm DNA fragmentation, human spermatogenesis, testicular spermatogenic cells, Pathology Section

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ABSTRACT

We performed immunofluorescent analysis of DNA hydroxymethylation and methylation in human testicular spermatogenic cells from azoospermic patients and ejaculated spermatozoa from sperm donors and patients from infertile couples. In contrast to methylation which was present throughout spermatogenesis, hydroxymethylation was either high or almost undetectable in both spermatogenic cells and ejaculated spermatozoa. On testicular cytogenetic preparations, 5-hydroxymethylcytosine was undetectable in mitotic and meiotic chromosomes, and was present exclusively in interphase spermatogonia Ad and in a minor spermatid population. The proportions of hydroxymethylated and non-hydroxymethylated diploid and haploid nuclei were similar among samples, suggesting that the observed alterations of 5-hydroxymethylcytosine patterns in differentiating spermatogenic cells are programmed. In ejaculates, a few spermatozoa had high 5-hydroxymethylcytosine level, while in the other ones hydroxymethylation was almost undetectable. The percentage of highly hydroxymethylated (5-hydroxymethylcytosine-positive) spermatozoa varied strongly among individuals. In patients from infertile couples, it was higher than in sperm donors ($P<0.0001$) and varied in a wider range: 0.12-21.24% versus 0.02-0.46%. The percentage of highly hydroxymethylated spermatozoa correlated strongly negatively with the indicators of good semen quality – normal morphology ($r=-0.567$, $P<0.0001$) and normal head morphology ($r=-0.609$, $P<0.0001$) – and strongly positively with the indicator of poor semen quality: sperm DNA fragmentation ($r=0.46$, $P=0.001$). Thus, the immunocytochemically detected increase of 5hmC in individual spermatozoa is associated with infertility in a couple and with deterioration of sperm parameters. We hypothesize that this increase is not programmed, but represents an induced abnormality and, therefore, it can be potentially used as a novel indicator of semen quality.

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Catechol-O-methyltransferase Val158Met polymorphism is associated with increased risk of multiple uterine leiomyomas either positive or negative for *MED12* exon 2 mutations

Lyailya Kh Dzhemlikhanova,[1,2] Olga A Efimova,[1,2] Natalia S Osinovskaya,[1] Sergey E Parfenyev,[2] Dariko A Niauri,[1,2] Iskender Yu Sultanov,[1] Olga V Malysheva,[1] Anna A Pendina,[1,2] Natalia Yu Shved,[1] Tatyana E Ivashchenko,[1] Maria I Yarmolinskaya,[1] Maka I Kakhiani,[1] Ekaterina A Gorovaya,[2] Antonina N Tkachenko,[3] Vladislav S Baranov[1,2]

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[1]D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russia
[2]St. Petersburg State University, St. Petersburg, Russia
[3]Maternity Hospital of St. Petersburg №6, St. Petersburg, Russia

Correspondence to
Dr Olga A Efimova, D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Mendeleevskaya line, 3, St. Petersburg 199034, Russia; efimova_o82@mail.ru

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ABSTRACT

Aims To study the possible association of catechol-O-methyltransferase (*COMT*) Val158Met polymorphism with multiple and solitary uterine leiomyomas (ULs) and to check whether the *COMT* Val/Val genotype is associated with *MED12* exon 2 mutations in fibroids.

Methods The *COMT* Val158Met allele and genotype frequencies were compared between age-matched women with ULs (n=104) and controls (n=59). Patients with UL were subcategorised by diagnosis of solitary (n=59) or multiple (n=45) fibroids and by the presence of somatic *MED12* exon 2 mutations in at least one fibroid (n=32) or in neither fibroid (n=26). The association of *COMT* Val/Val genotype with the presence of any ULs, solitary/multiple ULs and ULs positive/negative for *MED12* exon 2 mutations was evaluated by χ^2 tests using a dominant genotype model (G/G vs G/A+A/A) and expressed as ORs and 95% CIs.

Results The *COMT* Val/Val genotype frequency did not differ between the patients with UL and the controls (28.8% vs 18.6%, p=0.149, OR 1.77; CI 0.81 to 3.86). However, it was significantly higher in the patients who had multiple UL compared with the solitary UL (40% vs 20.3%, p=0.028, OR 2.61; CI 1.09 to 6.24) and to the controls (40% vs 18.6%, p=0.016, OR 2.91; CI 1.20 to 7.06). No association of the *COMT* Val/Val genotype with UL-specific *MED12* exon 2 mutations was found (p=0.662, OR 0.77; CI 0.23 to 2.53).

Conclusions Women with *COMT* Val/Val genotype are at high risk of developing multiple uterine fibroids either positive or negative for *MED12* exon 2 mutations. These data are important to design new strategies for UL prophylaxis and treatment.

INTRODUCTION

Uterine leiomyoma (UL) (also known as uterine fibroid) is the most common benign tumour of uterus with an incidence over 70% in women of reproductive age.[1] UL is a multifactorial disease; its origin and development are driven by both exogenous and endogenous factors. A number of clinical and epidemiological observations suggest that gene and/or chromosomal alterations play a significant role in the UL development.[2 3] Among UL-specific gene abnormalities, the most common are those affecting the *MED12* (mediator subcomplex 12), the *HMGA2* (high mobility group A 2) or the *FH* (fumarate hydratase) gene.[4] However, it remains not clear whether they initiate tumour growth or represent secondary alterations in already affected myocytes.

The numerous data indicate that sex steroid hormonal status and exposure are among well-recognised factors predisposing to UL.[5] Catechol-O-methyltransferase (COMT) is involved in metabolism of steroid hormones; it catalyses transfer of a methyl group from S-adenosylmethionine (SAM) to a hydroxyl group of dopamines, norepinephrines or catechol oestrogens.[6] The G to A substitution at codon 158 is a common *COMT* polymorphism (also known as G472A or Val158Met), resulting in incorporation of methionine instead of valine and in significant decrease in enzyme activity.[7] The data on the high-activity *COMT* Val/Val genotype association with an increased risk of UL development are controversial.[8–16]

Here, we studied *COMT* Val158Met polymorphism in patients with solitary and multiple ULs and checked whether it is associated with *MED12* exon 2 mutations in uterine fibroids.

MATERIALS AND METHODS

Studied groups

Two groups of individuals were enrolled in the study: women diagnosed with UL (UL group, n=104) and randomly selected women (control group, n=59). The age ranged from 24 to 59 years (mean 40.4±0.8) in the UL group and from 24 to 61 years (mean 41.1±1.4) in the control group. All the individuals were residents of North-West of Russian Federation and were of the Russian ethnicity.

All the patients with UL underwent hysterectomy or myomectomy in D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, St. Petersburg, Russia. Indications for surgery included rapid UL growth, pregnancy planning, infertility, chronic pelvic pain syndrome. The diagnosis of UL—solitary or multiple—was based on expert ultrasound examination of the pelvic organs prior surgery and histological analysis of the removed specimen after surgery. Of 104 patients with UL, 59 had solitary ULs (solitary UL subgroup) and 45 had multiple

Raising confidence threshold increases the positive predictive value of a SNP-based NIPT for the 22q11.2 microdeletion

A. Ryan, S. Iyengar, B. Jiorle, Z. Demko;
Natera Inc, San Carlos, CA, United States.

AIM: To examine whether raising the confidence threshold at which the algorithm for a SNP-based NIPT for the 22q11.2 microdeletion makes a positive call would increase the positive predictive value (PPV) without affecting test sensitivity.
METHODS: 20,776 NIPT samples received between Feb-Aug, 2014 were previously evaluated for the 22q11.2 deletion using primers targeting 672 SNPs in a 2.9 Mb segment of 22q11.2, and analyzed using a high-risk confidence threshold of 0.90. Follow-up information, including ultrasound findings, were collected for high-risk cases. Here, the algorithm's confidence threshold was raised to 0.95, and PPV recalculated for the entire cohort and for the subset of cases with prior-known directly-associated ultrasound anomalies (high a priori risk) and with no prior-known anomalies (low a priori risk).
RESULTS: At the original confidence cut-off, the test had a PPV of 18%, which increased to 42.3% upon reflex-sequencing of high-risk samples at a higher read depth (HDOR). Raising the algorithm's confidence level increased the PPV to 52.4% and reduced the false positive rate from 0.12% to 0.07%, with no loss in test sensitivity. The PPV was 100% for high a priori risk cases, and 20% for low a priori risk cases.
CONCLUSIONS: The updated methodology (raised confidence level + reflexing) eliminated 80% of the FP cases originally reported, yielding an improved PPV for this SNP-based NIPT (52.4% vs. 18%). Given the higher than previously-reported incidence of the 22q11.2 microdeletion and the benefits of early intervention, we expect this improved test to be highly beneficial in prenatal testing.

P01.003
5-methylcytosine and 5-hydroxymethylcytosine patterns in human spermatogenic cells

A. V. Tikhonov[1,2], O. A. Efimova[1,2], A. A. Pendina[1,2], I. D. Fedorova[2], S. E. Parfenyev[1], A. S. Koltsova[1,2], T. V. Kuznetzova[1,2], V. S. Baranov[1,2];
[1]Saint Petersburg State University, St.Petersburg, Russian Federation, [2]D.O.Ott Research Institute of Obstetrics, Gynecology and Reproductology, St.Petersburg, Russian Federation.

We aimed to study 5-hydroxymethylcytosine (5hmC) and 5-methylcytosine (5mC) patterns in human spermatogenic cells. Samples were obtained by testicular biopsy of 15 patients diagnosed with azoospermia. Chromosome and nuclei preparations were made by "direct" technique using hypotonic (0.9% sodium citrate) and colchicines treatment and fixation with methanol:acetic acid, 3:1. Using indirect immunofluoresecence, we have analyzed the localization of 5hmC and its co-distribution with 5mC in the nuclei and chromosomes from spermatogenic cells. To visualize nuclei and to identify chromosomes, we used QFH/AcD-staining. To determine nucleus ploidy, we used FISH with two centromeric DNA-probes.
5mC was detected in all nuclei from both diploid and haploid cells and in all chromosomes from both mitotic spermatogonia and meiotic spermatocytes. 5hmC showed a different pattern: it was present only in some nuclei and was totally absent in mitotic and meiotic chromosomes. By comparing hydroxymethylation and FISH patterns in 5000 nuclei from each sample, we established that 5hmC was present in 32-52% of diploid nuclei from spermatogonia and Sertoli cells. In contrast, among haploid nuclei from spermatids, only 0.6-1.9% was hydroxymethylated.
Thus, in contrast to 5mC, global 5hmC pattern changes during human spermatogenesis. The presence of 5hmC in a minor set of spermatids suggests active demethylation of their genome. The fate of these hydroxymethylated spermatids during spermiogenesis is to be elucidated in further studies.
Supported by RFBR (16-34-00532_mol_a).

P01.004
Application of a-CGH for preimplantation genetic testing (PGT) in young women and patients of advanced maternal age

O. A. Yacuts, J. V. Tsukerman, A. B. Zhabinskaya, A. V. Aliakseyeva, O. L. Tishkevich;
Centre of assisted reproduction «Embryo», Minsk, Belarus.

PGT was started in our center in 2012 with FISH diagnostics. In 2015 we began applying array comparative genomic hybridization (a-CGH) for preimplantation screening and diagnostics. Indications for PGT included: advanced maternal age (AMA) (>35), repeated implantation failure (>2), balanced chromosome rearrangements.
Materials and Methods: We conducted 14 cycles, IVF+ICSI. Patients were divided into 2 cohorts: 1) young patients (<35) and 2) AMA (>35). Average age was 29,5 ±1,4 and 41,2±2,2, respectively. A-CGH (Bluegnome, Illumina) was used to detect whole chromosome aneuploidies. Trophectoderm biopsy was performed on 5-6 day of embryo development. Blastocysts were vitrified and transferred in next cycle after PGT.
Results:
PGT was performed for 48 blastocysts and aneuploidy rates were 53,1% for young patients and 100% for AMA group. The reason for high aneuploidy rate in AMA group is age and small number of blastocysts. Complex chromosome abnormalities were more common in AMA group (Table 1). The most frequent aneuploidies were trisomies (37,5%) and monosomies (35,4%), Aneuploidies for X and Y chromosomes were detected in 8,3%.

	Young patients	AMA
Average number of blastocysts per cycle	4	2,7
Euploid blastocysts,%	46,9	0
Blastocysts with complex chromosome abnormalities,%	15,6	31,2
Failed amplification,%	4,2	

Conclusion: In cohort of AMA the average number of blastocysts on day 5 and 6 was significantly lower than in patients of younger age. Complex chromosomal aberrations were double higher in this group.

P01.005
Male age is not related with high rates of spermatozoa and embryos aneuploidy

Immunofluorescence Staining for Cytosine Modifications Like 5-Methylcytosine and Its Oxidative Derivatives and FISH

Anna A. Pendina, Olga A. Efimova, Andrei V. Tikhonov, Olga G. Chiryaeva, Irina D. Fedorova, Alla S. Koltsova, Mikhail I. Krapivin, Sergey E. Parfenyev, Tatyana V. Kuznetzova, and Vladislav S. Baranov

Abstract

In this chapter, the protocol of combined immunofluorescence detection of 5-methylcytosine and its oxidative derivatives and FISH is given. This approach can be applied on fixed preparations of human chromosomes and nuclei. Human PHA-stimulated adult and fetal lymphocytes, uncultured tissues comprising spontaneously dividing cells, such as chorion, embryonic and testicular tissues (prepared using "direct" technique), oocytes, zygotes and blastomeres of preimplantation human embryos, and non-cultured uterine leiomyoma samples may be used. The combined immunostaining for DNA methylation allows simultaneous assessment of the whole-genome methylation pattern as well as of genomic subunits, thus indicating the functional status of nuclei with different karyotypes; it is also suited for homologous or structurally abnormal chromosomes characterizable by FISH. Combined immunostaining/FISH is an indispensable method for investigation of both programmed and abnormal de novo alterations of DNA methylation.

Keywords DNA methylation, 5-methylcytosine, 5-hydroxymethylcytosine, FISH, Marker chromosomes, Nuclei, Human karyotype, Immunofluorescence staining, Direct chromosome preparation

1 Introduction

In 1974, Miller and coworkers published in *Nature* their immunofluorescence-based studies of DNA methylation patterns in murine and human metaphase chromosomes, reporting heterochromatin-specific location of 5-methylcytosine (5mC) residues [1]. Their results initiated a new direction of research, focusing not only on methylated DNA extracted from cells but also on study of DNA methylation in situ, directly on metaphase chromosomes. With the discovery of DNA methylation as a mechanism of cell memory [2, 3] and as a major epigenetic mechanism regulating gene activity and chromatin structure ([4]; chapter by Tiphaine

Thomas Liehr (ed.), *Fluorescence In Situ Hybridization (FISH)*, Springer Protocols Handbooks,
DOI 10.1007/978-3-662-52959-1_35,

Приложение 2

Участие в научных конференциях, семинарах и т.п.

Размещаются соответствующие копии документов

Приложение 3.

Дополнительная информация

Размещаются соответствующие копии документов