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CELLULAR REPROGRAMMING - THE 2012 NOBEL PRIZE IN PHYSIOLOGY OR MEDICINE

REPROGRAMIRANJE ĆELIJA - NOBELOVA NAGRADA ZA FIZIOLOGIJU ILI MEDICINU ZA 2012. GODINU

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Abstract

Differentiated cells can be reprogrammed to become pluripotent stem cells capable of developing into other types of mature cells. First experiments involved replacement of the cell nucleus of a frog's egg cell with a nucleus from a specialised somatic cell. Later on, immature stem cells were obtained by introducing only several genes into differentiated cells, which provided new tools for diagnosis and therapy.

Key words

Nobel Prize in physiology or medicine; cell reprogramming; induced pluripotent stem cells.

Apstrakt

Diferencirane ćelije se mogu in vitro reprogramirati tako što im se menja ekspresija gena, pa se od jednog tipa ćelija može dobiti drugi, nesrodan tip ćelija. Prva istraživanja iz ove oblasti rađena su prebacivanjem nukleusa somatskih ćelija žaba u njihove jajne ćelije. Direktnim reprogramiranjem diferenciranih ćelija miša, a zatim i humanih ćelija, pomoću regulatora ekspresije gena, dobijene su indukovane pluripotentne ćelije koje se mogu primeniti za zamenu oštećenih ćelija ili tkiva kod raznih oboljenja.

Ključne reči

Nobelova nagrada za fiziologiju ili medicinu; reprogramiranje ćelija; indukovane pluripotentne matične ćelije.

This year Sir John B. Gurdon, a British developmental biologist from Gurdon Institute in Cambridge and Shinya Yamanaka, a Japanese physician, currently a professor at *Kyoto University*, senior investigator at the Gladstone Institute in San Francisco and professor of anatomy at *University of California*, were awarded the Nobel Prize in physiology or medicine. They are awarded for their life-long investigation of cell development and the discovery that mature cells can be reprogrammed and converted to embryonic cells capable of generating any cell and tissue type of the body.

John Gurdon was first of them who started the investigation in this field. In the begining of his career he was investigating formation of specialized cells during development from fertilized egg into adult organism. In experiments with frogs, which we now consider as classic, Gurdon transplanted nucleus from fully differenciated intestinal cell into enucleated egg cell. Entirely normal and fertile male and female frogs were obtained [1]. Before that, in similar experiments of other authors, the transfer of nuclei from slightly older embryos, in the stage of gastrula, into an egg cell, resulted only in abnormal development, and they concluded that cell differentiation was likely to involve irreversible nuclear changes [2]. In contrast, Gurdon concluded that the process

Ove godine Nobelovu nagradu za fiziologiju ili medicinu dobili su Sir John B. Gurdon, engleski biolog iz Gurdon Institute, Cambridge i Shinya Yamanaka, japanski lekar koji trenutno radi kao profesor na *Kyoto University*, kao istraživač na Gladstone Institute, San Francisco, California i kao profesor anatomije na *University of California*, San Francisco. Nagrađeni su za dugogodišnja istraživanja razvića ćelija i organizama i otkriće da zrele, specijalizovane ćelije mogu da se reprogramiraju i postanu nezrele ćelije sposobne da se razviju u bilo koju ćeliju ili tkivo u organizmu.

Istraživanja iz ove oblasti mnogo ranije je započeo John Gurdon. Na početku svoje karijere on je ispitivao postepenu specijalizaciju ćelija tokom razvića od oplođene jajne ćelije do odraslog organizma. U eksperimentima sa žabama, koji se sada smatraju klasičnim, Gurdon je nezreloj jajnoj ćeliji zamenio nukleus sa nukleusom iz zrele, intestinalne ćelije. Ovako modifikovana jajna ćelija razvila se u normalnog punoglavca [1]. Prethodna slična ispitivanja drugih autora, kod kojih su prebačeni nukleusi nešto starijeg embriona, iz faze gastrule u jajnu ćeliju, dovela su do abnormalnog razvića, pa su autori zaključili da se tokom diferencijacije ćelija verovatno dešavaju ireverzibilne promene u nukleusu [2]. Za razliku od njih, Gurdon je zaključio da se proces diferencijacije ćelija može potpuno obrnuti i da on ne zahte-

of cell differentiation can be fully reversed and does not require irreversible nuclear changes. In the course of differentiation, changes occur in gene expression but not in the gene content itself. Cells become stably and functionally very different from each other during development, but their genome stays the same in all cells and therefore retains the potential to form any cell type. The only exception are antibody-producing cells that lose segments of their genome during maturation.

Gordon's discoveries initiated intense research which led to the cloning of mammals and production of a normal adult sheep (Doli) by transplating the nuclei of cultured mammary gland cells derived from an adult sheep to enucleated sheep eggs [3]. This suggested that similar procedure might be applied to humans as well.

In the later stage of his investigation, Gordon examined if the process of mammal cell differentiation can be reversed to pluripotent state. A skin cell does not naturally give rise to a brain cell, nor does an intestine cell generate a heart cell. But using certain experimental procedures Gurdon wanted to achieve just this: to switch the nuclear gene expression of one cell type to that of an embryo or another cell type. This process is called nuclear reprogramming. It allows us to understand how cell differentiation is normally maintained and how gene expression is controled. Beside this, nuclear reprogramming has a practical application in regenerative medicine [4]. Defective cells or tissues can be replaced by normal cells of the same kind derived from a different cell type by the process of nuclear reprogramming. If these cells belong to the same individual, the need for immunosuppression, that othervise must be applied, is avoided.

For Gurdon and his assistants it was clear that egg cytoplasm has remarkable potential of reprogramming somatic cell nuclei, but they did not know what molecules and mechanisms are involved in reversing the gene expression pattern of a differentiated cell into an embryonic state in just a few hours.

His examination of pluripotent cells, that are called stem cells, Yamanaka started at the Nara Institute of Science and Technology, and production of induced pluripotent stem cells at the Institute for Frontier Medical Sciences, *Kyoto University*. His idea was to produce stem cells by reprogramming mature cells in culture. Yamanaka and his team performed first experiments with adult mouse fibroblasts. By inserting certain molecules in fibroblasts they generated pluripotent cells that they called induced pluripotent stem cells (iPS cells). These cells resemble embrionic stem cells, the *in vitro* equivalent of the part of the blastocyst, the embryo of a few days [5]. Yamanaka could show that his iPS cells were pluripotent, i.e. that were capable of developing into all cell lineages of the body, including an adult mice. Generating of iPS cells from adult cells, as Yamanaka started, is significant because it offered a way of obtaining human stem cells avoiding the controversal use of human embryos.

In the course of reprograming of adult cells, Yamanaka introduced certain genes that were known to be the main regulators of gene expression. These genes were *Oct3/4, Sox2, Klf4*, and *c-Myc*. Introduction of these genes into the genome of adult cells was achieved by retrovirus. It was assumed that *c-Myc* plays a fundamental role in reprogram-

va ireverzibilne nuklearne promene. Tokom diferencijacije se dešavaju promene u ekspresiji gena, a ne u njihovom sastavu. Ćelije postaju funkcionalno različite i ta sudbina im je trajna i stabilna, ali njihov genom ostaje isti u svim ćelijama, tako da one zadržavaju potencijal da formiraju bilo koji tip ćelije određenog organizma. Izuzetak predstavljaju samo ćelije koje produkuju antitela, koje u određenoj fazi sazrevanja gube pojedine delove genoma.

Istraživanja Gurdona omogućila su kasnije eksperimente kloniranja organizama. Najpoznatiji primer je produkcija normalne odrasle ovce (Doli), koja je nastala transplantacijom nukleusa iz kultivisanih ćelija mlečne žlezde odrasle ovce u jajnu ćeliju ovce iz koje je uklonjen nukleus [3]. Ovim je nagovešteno da bi se slična procedura mogla primeniti i kod ljudi.

U kasnijoj fazi svog rada Gurdon je ispitivao da li se proces diferencijacijacije ćelija sisara može obrnuti i iskoristiti njihov pluripotentni potencijal. Od ćelija kože nikada prirodno ne nastaju ćelije mozga, niti od intestinalnih ćelija nastaju ćelije srca. Međutim, Gurdon je određenim eksperimentalnim procedurama želeo da postigne upravo to: da preusmeri ekspresiju gena tako da od jednog tipa specijalizovanih ćelija dobije embrionalne ćelije ili drugi tip ćelija. Ovaj proces se naziva nuklearnim reprogramiranjem. Osim što nam omogućava da razumemo kako se odvija diferencijacija ćelija i kako se kontroliše ekspresija gena tokom diferencijacije, nuklearno reprogramiranje ima praktičnu vrednost i može se primenjivati u regenerativnoj medicini [4]. Obolele ćelije ili tkivo mogu se zameniti normalnim ćelijama iste ili slične vrste, koje se mogu dobiti od različitih tipova ćelija nuklearnim reprogramiranjem. Ako ove ćelije pripadaju oboleloj osobi, izbegava se primena imunosupresivne terapije, koja se mora primenjivati kada se ćelije prenose između osoba koje su genetički različite.

Gurdonu i njegovim saradnicima je bilo jasno da citoplazma jajne ćelije ima kapacitet da reprogramira nukleuse somatskih ćelija, ali im nije bilo poznato koje molekule i koji mehanizmi su uključeni u reverziju ekspresije gena diferenciranih ćelija, koje samo za nekoliko sati dolaze do embrionalnog stanja.

Yamanaka je ispitivanja pluripotentnih ćelija, koje su nazvane matičnim ćelijama (stem cells), započeo na Nara Institute of Science and Technology, a nakon toga dobijanje indukovanih pluripotentnih matičnih ćelija na Institute for Frontier Medical Sciences, *Kyoto University*. Njegova ideja je bila da se matične ćelije dobiju tako što će se u kulturi, bez posredstva jajne ili embrionalne ćelije, reprogramirati adultne ćelije i dovesti do faze pluripotentnih matičnih ćelija, sličnih embrionalnim pluripotentnim ćelijama. Yamanaka i njegov tim su prve eksperimente radili sa fibroblastima odraslog miša. Ubacivanjem određenih molekula u fibroblaste dobili su pluripotentne ćelije koje su nazvali indukovane pluripotentne matične ćelije (iPS ćelije). One su odgovarale embrionalnim matičnim ćelijama i bile *in vitro* ekvivalent blastocistu ili embrionu od nekoliko dana [5]. Pluripotentne ćelije se, zatim mogu usmeriti da sazru u bilo koji tip diferenciranih ćelija u organizmu, ili se od njih mogao dobiti odrasli miš. Dobijanje iPS ćelija od adultnih ćelija, kako je zamislio Yamanaka, je značajno zbog toga što eliminiše potrebu da se za njihovo dobijanje koriste embrioni, što je ranije bilo povezano sa brojnim etičkim i drugim problemima.

ming the nuclei of adult cells. However its activation during the proces of generating iPS cells led to the formation of tumours when the stem cells were later transplanted into mice.

In 2007, Yamanaka performed similar experiment with human adult fibroblasts [6]. The cells were treated with the same four DNA fragments as in experiments with adult mouse fibroblasts, and these factors are now called Yamanaka factors. Human iPS cells were similar to human embryonic stem cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity. Telomerases are enzymes expressed during embryonal development, which maintain the length of chromosomes during replication. For the discovery of telomerase the Nobel Prize was awarded in 2009 [7].

Yamanaka subsequently created iPS cells without *c-Myc* in order to render the cells noncancerous and thereby overcome a major concern in the therapeutic safety of iPS cells. Remaining problem was the use of retroviruses to insert the genes into the nuclei because these viruses can also cause mutations in the adult cells, making them cancerous. The next challenge for Yamanaka's team and other reseachers all over the world is to find an alternative way of reprogramming the cells without the use of retroviruses.

The groundbraking findings of this year's Nobel Prize winners have provided new opportunities to reprogram mature cells from patients and study disease. These discoveries revolutionised the use of stem cells as tools for disease therapy and regenerative medicine.

Da bi reprogramirao adultne ćelije, Yamanaka im je dodavao gene koji su bili glavni regulatori ekspresije drugih gena. To su bili *Oct3/4, Sox2, Klf4,* i *c-Myc*. U genom adultne ćelije unosio ih je pomoću retrovirusa. Za *c-Myc* gen se verovalo da ima ključnu ulogu u reprogramiranju nukleusa adultnih ćelija. Međutim, njegova aktivacija tokom dobijanja iPS ćelija, dovodila je do formiranja tumora kada su matične ćelije kasnije transplantirane u miševe.

2007. godine Yamanaka je sličan eksperiment ponovio sa adultnim humanim fibroblastima [6]. Ćelije je tretirao sa ista četiri fragmenta DNK kao u eksperimentima sa adultnim ćelijama miša, pa se oni sada po njemu nazivaju Yamanaka faktori. Humane iPS ćelije koje je dobio, bile su slične humanim embrionalnim matičnim ćelijama po morfologiji, proliferaciji, površinskim antigenima, ekspresiji gena, epigenetskom statusu gena specifičnih za pluripotentne ćelije i po aktivnosti telomeraze. Telomeraze su enzimi koji se sintetišu u početnoj fazi razvića organizama, kada omogućavaju održavanje dužine krajeva hromozoma tokom njihove replikacije. Za istraživanje telomera i telomeraza dodeljena je Nobelova nagrada 2009. godine [7].

Usavršavajući metodu dobijanja iPS, Yamanaka je radio na tome da smanji rizik od nastajanja tumora nakon primene ovih ćelija u terapiji. Za indukciju više nije primenjivao *c-Myc* gen, koji je bio poznat onkogen. Problem je predstavljala i primena retrovirusa za inserciju gena u hromozome ćelija, zato što je za ove viruse poznato da mogu izazvati mutacije u adultnim ćelijama i dovesti do pojave tumora. Zbog toga je sledeći izazov za Yamanaku i njegove saradnike, kao i za mnoge druge istraživače širom sveta koji se bave istraživanjima vezanim za reprogramiranje ćelija, da nađu alternativni način da se geni efikasno ubace u genom ćelije bez primene retrovirusa.

Radovi dvojice ovogodišnjih dobitnika Nobelove nagrade omogućavaju dobijanje matičnih ćelija iz adultnih ćelija samog pacijenta, a one mogu biti specifične i za samo oboljenje. Time je načinjen veliki korak u mogućnosti praktične primene matičnih ćelija u terapiji raznih oboljenja i u regenerativnoj medicini.

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