Statistički testovi procjene rizika

Risk reduction statistics

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Sažetak

Statistički testovi procjene rizika (engl. *risk reduction statistics*) su skupina statističkih testova koji se sve češće rabe u kliničkoj praksi uslijed sve učestalije primjene prakse temeljene na dokazima u svrhu kliničke njege bolesnika. Njihova se uporaba temelji na spoznaji da se sva liječenja i svi lijekovi prepisuju kako bi se smanjio rizik od neželjenog ishoda kod bolesnika. Niti za jedno se liječenje ne može garantirati da će u potpunosti izliječiti bolesnika. Kao rezultat toga, publikacije, koje objavljuju informacije o najboljim praksama liječenja, sve češće nude logičnu podlogu za donošenje odluka u vezi liječenja u kontekstu smanjenja rizika za pojedinog bolesnika i populaciju bolesnika. Najčešće rabljeni statistički testovi procjene rizika su apsolutno smanjenje rizika (engl. *absolute risk reduction*, ARR) i relativno smanjenje rizika (engl. *relative risk reduction*, RRR). Određivanje apsolutnog smanjenja rizika je jednostavan postupak u kojem se od rizika od neželjenog ishoda bez liječenja oduzima rizik od neželjenog ishoda s liječenjem. Test relativnog smanjenja rizika je kompleksniji statistički test koji izračunava smanjenje rizika kod liječenih bolesnika u odnosu na rizik kod neliječenih bolesnika. Oba statistička testa mogu pomoći liječnicima i bolesnicima dovesti u ravnotežu smanjenje rizika od neželjenog ishoda s troškovima i nuspojavama predloženog liječenja.

Ključne riječi: statistika; epidemiologija; apsolutno smanjenje rizika; relativno smanjenje rizika; klinička statistika

Abstract

Risk Reduction statistics are a group of statistics that are increasingly used in clinical practice as more practitioners use evidence-based practice as their approach to clinical care. Their use involves the recognition that all treatments are prescribed to reduce the patient's risk of an adverse outcome. No treatment can be guaranteed to completely cure all patients. As a result, papers presenting information on best treatment practices are increasingly presenting the rationale for treatment decisions in the context of risk reduction for individual patients and patient populations. The most commonly used risk reduction statistics are Absolute Risk Reduction and Relative Risk Reduction. Absolute risk reduction is simply the risk of an adverse outcome with no treatment less the risk of an adverse outcome with treatment. Relative risk reduction is a more complex statistic that calculates risk reduction for treated patients relative to the risk for untreated patients. Both statistics can be used to assist practitioners and patients to balance the reduction in risk for an adverse event against the cost and side-effects of the proposed treatment.

Key words: statistics; epidemiology; absolute risk reduction; relative risk reduction; clinical statistics

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Testovi procjene rizika

Testovi procjene rizika sve su važniji liječnicima i medicinskim sestrama u praksi primarne zdravstvene zaštite. Kao rezultat toga, publikacije, koje objavljuju informacije o najboljim praksama liječenja, sve češće nude logičnu podlogu za donošenje odluka u vezi liječenja u kontekstu smanjenja rizika za pojedinog bolesnika i populaciju bolesnika. Taj se trend može pripisati dvama faktorima. Prvi je da je dobro poznato kako se niti od jednog liječenja ne može očekivati željeni ishod kod svakog bolesnika. Drugi jest da testovi procjene rizika omogućuju liječniku dobi-

Risk reduction statistics

Risk reduction statistics have become increasingly important to physicians and nurses in primary care practice. As a result, papers presenting information on best treatment practices are increasingly presenting the rationale for treatment decisions in the context of risk reduction for individual patients and patient populations. This trend can be attributed to two factors. First, it is well known that no treatment can be expected to produce the desired outcome for every patient. Second, risk reduction statistics allow the practitioner to understand the proba-

ti uvid u vjerojatnost uspješnosti liječenja kod bolesnika te da mu pomažu objasniti koji se ishodi liječenja mogu očekivati na način da se objasne vjerojatnosti za svaki ishod. Na posljetku, same odluke o liječenju temelje se na vjerojatnosti hoće li liječenje smanjiti vjerojatnost nepovoljnog ishoda. Primjerice, ako je djetetu dijagnosticirana leukemija, liječnik će roditelju željeti prezentirati različite mogućnosti liječenja i objasniti stupanj u kojem će svaka od tih mogućnosti smanjiti rizik od smrti od te bolesti. Na taj način i liječnik i roditelj razumiju potencijalne rizike i koristi svakog mogućeg pristupa liječenju. Testovi procjene rizika dio su skupine statističkih testova koji potječu iz epidemiološkog koncepta te su korisni u praksi temeljenoj na dokazima budući da mogu služiti kao pomoć liječnicima u donošenju odluke je li vjerojatno da će pojedini bolesnik imati koristi od određene intervencije liječenjem (3).

Testovi procjene rizika u biti izvještavaju o razlici između vjerojatnosti (rizika) neželjenog ishoda kod neliječenog bolesnika i vjerojatnosti istog ishoda kod bolesnika koji je na određenom liječenju. Statistički testovi procjene rizika koji se najčešće rabe u kliničkoj praksi kod odlučivanja o liječenju su apsolutno smanjenje rizika (engl. *absolute risk reduction*, ARR) i relativno smanjenje rizika (engl. *relative risk reduction*, RRR). Kliničari trebaju također razumjeti i statistički postupak određivanja broja ispitanika koje je potrebno liječiti (engl. *number needed to treat*, NNT). NNT obično pojašnjava što znači statistički test procjene rizika. Koncept procjene rizika potječe iz epidemiologije i javnog zdravstva. Ti se testovi temelje na iskustvu s populacijom. U donošenju odluke o liječenju pomoću tih testova vrlo je važno da kliničar bude svjestan populacije na kojoj su ti testovi napravljeni i u kojoj mjeri pojedinac kojeg treba liječiti pripada toj populaciji.

Apsolutno smanjenje rizika

Kada kliničar prepiše lijek u svrhu liječenja bolesti namjera mu je ili izliječiti tu bolest ili smanjiti vjerojatnost da će se pojaviti sekundarni učinci te bolesti. Primjerice, kada se septičnog bolesnika liječi antibioticima, cilj je izliječiti ga. Drugim riječima, svrha antibiotika je smanjiti rizik da bolesnik umre od infekcije. Kod skupine bolesnika s kroničnim bolestima liječenja se prepisuju kako bi se smanjio rizik od invaliditeta i pojave sekundarne bolesti srodne toj kroničnoj bolesti. Primjerice, bolesnicima s reumatoidnim artritisom prepisuju se lijekovi kako bi se smanjio rizik od daljnje deformacije zglobova, bol i rizik od smanjenja bolesnikove sposobnosti izvršavanja aktivnosti svakodnevnog života (engl. *activities of daily living*, ADLs). Poznato je da neki bolesnici bolje reagiraju na liječenje nego drugi, a o statističkim testovima koji govore o ishodima liječenja za cjelokupnu populaciju može se raspravljati jedino u smislu stupnja do kojeg liječenje smanjuje rizik od neželjenog ishoda.

Najjednostavniji statistički postupak određivanja procjene rizika jest određivanje apsolutnog smanjenja rizika. Za

bility of treatment success for patients, and to explain to patients what outcomes can be expected in terms of the probability of each outcome. Treatment decisions ultimately are based on the likelihood that the treatment will reduce the probability of a poor outcome. For example, if a child is diagnosed with leukemia, the physician may want to present to the parent the various treatment options and explain the degree to which each option will reduce the risk of death from that disease. In that way, the physician and parent both understand the potential risks and benefits of each possible treatment approach. Risk Reduction statistics are part of a group of statistics emerging from epidemiology concepts that have been found useful in evidence-based practice because they can be used to assist practitioners to decide whether or not an individual patient is likely to benefit from a particular treatment intervention (3).

Risk statistics essentially report the difference between the likelihood (risk) of an undesirable outcome for an untreated patient, and the likelihood of that outcome for a patient that receives a particular treatment intervention. The risk reduction statistics most commonly used in clinical practice treatment decisions are “absolute risk reduction” and “relative risk reduction”. In addition, clinicians need to understand the statistic, “number needed to treat”. The “number needed to treat” statistic typically gives clarity of meaning to the risk reduction statistics. The concept of risk reduction emerged from epidemiology and public health. The risk reduction statistics are based on experience with populations. When the clinician uses these statistics to inform treatment decisions, it is important that the clinician understands the population upon which the statistics were reported, and the extent to which the individual being treated fits into that population.

Absolute risk reduction

When a clinician prescribes a drug to treat an illness, the purpose is to either cure the illness or to reduce the likelihood that a secondary effect of the illness will occur. For example, when a septic patient is treated with an antibiotic, the goal is to cure the patient. Another way to view that goal is that the purpose of the antibiotic is to reduce the patient’s risk of death from the infection. In the chronic illness population, treatments are prescribed to reduce the risk of disability and secondary illnesses related to the chronic illness. For example, medications are prescribed to patients with rheumatoid arthritis to reduce the risk of further joint deformity, pain and the risk of a decrease in the patient’s ability to perform activities of daily living (ADLs). It is known that some patients will respond better to treatment than others, and the overall population statistics related to treatment outcomes can be discussed in terms of the extent to which the treatment reduces the risk of an adverse outcome.

The simplest risk reduction statistic is absolute risk reduction. The calculation of this statistic requires two items of

primjenu ovog testa potrebno je znati dva podatka. Prvo nam mora biti poznat rizik od neželjenog ishoda (događaj) kod neliječene populacije. Primjerice, rizik od smrtnosti kod djece s leukemijom je približno 99% ako se ne liječe. (Važno je da mlađi znaju da je prije početka suvremenog liječenja leukemije, doslovce svako dijete zahvaćeno tom bolešću umrlo u roku od oko 3 mjeseca. Čak sve do 50-ih i 60-ih godina prošlog stoljeća, kada je liječenje produljilo životni vijek većini bolesnika, veći je dio njih umro u roku od 5 godina, a doslovno svi su umrli u roku od 10 godina.) Stoga, rizik od smrtnosti u slučaju neliječenja od te bolesti iznosi oko 99%. Drugo, važno je znati sveukupni rizik u populaciji od smrtnosti u slučaju predloženog liječenja. Ako je dostupno više od jednog liječenja, tada mora biti poznat rizik od smrtnosti kod svakog pojedinog liječenja. Na primjer, 2005. je stopa smrtnosti od leukemije u dječjoj dobi u SAD bila 27,3%. 1990. je iznosila 34,2% (2). Apsolutno smanjenje rizika za liječenje leukemije u dječjoj dobi u 1990. bilo je 65,8% (100%–34,2%). Apsolutno smanjenje rizika u 2005. iznosilo je 72,7% (100%–27,3%). Izračun apsolutnog smanjenja rizika je jednostavan – rizik od smrtnosti kod liječene djece oduzme se od rizika od smrtnosti kod neliječene djece. Ili pojednostavljeno, apsolutno smanjenje rizika izračunava se kao originalni rizik (rizik kod neliječenih bolesnika) minus rizik kod liječenih bolesnika. Ako postoji više od jednog dostupnog liječenja, može se izračunati razlika u riziku između skupina liječenih svakim pristupom (tablica 1).

data. First, the risk of the undesirable outcome (event) in the untreated population must be known. For example, the risk of death for children with leukemia is approximately 99% if no treatment is provided. (It is important for younger individuals to know that prior to modern treatments for leukemia, virtually all children who contracted the disease died within approximately 3 months. Even up to the 1950s and 1960 when treatments extended life for most patients, most still died within 5 years and virtually all died within 10 years.) Therefore, the untreated risk of death from that disease is approximately 99%. Second, it is necessary to know the overall population risk of death given the proposed treatment. If more than one treatment is available, then the risk of death under each treatment condition must be known. For example, in 2005 the death rate from childhood leukemia in the U.S. was 27.3%. In 1990 it was 34.2% (2). The absolute risk reduction for childhood leukemia treatments in 1990 was 65.8% (100%–34.2%). The absolute risk reduction in 2005 was 72.7% (100%–27.3%). The absolute risk reduction statistic is simply the risk of death for treated children subtracted from the risk of death from untreated children. Or more generally, the statistic is calculated as the original (untreated) risk less the treated risk. Should there be more than one available treatment; the difference in risk between populations treated with each treatment can be calculated (see Table 1).

Tablica 1. Stopa događaja u skupini liječenih osoba, u kontrolnoj skupini i procjena rizika

Table 1. Experimental event rate, control event rate, and risk

		Outcome		
		Event (Stroke)	No-Event (No Stroke)	
Exposure	Treated	a = 3	b = 97	(a+b) = 100
	Control	c = 8	d = 92	(c+d) = 100
		(a+c) = 11	(b+d) = 189	

Experimental Event Rate (EER) – event rate in treated group = $\frac{a}{(a+b)} = \frac{3}{100} = 0.03$

Control Event Rate (CER) – event rate in control group = $\frac{c}{(c+d)} = \frac{8}{100} = 0.08$

CER is the risk of the event in the untreated population (8% in this example)

EER is the risk of the event in the treated population (3% in this example)

Formulae for RR, ARR, and RRR

Absolute Risk Reduction = {Absolute Value of CER – EER} = $|0.08 - 0.03| = 0.05$

$$\text{Relative Risk Reduction} = \frac{\text{Absolute Risk Reduction}}{\text{CER}} = \frac{0.05}{0.08} = 0.625 \text{ or } 62.5\%$$

$$\text{Relative Risk} = \frac{\text{EER}}{\text{CER}} = \frac{0.03}{0.08} = 0.375 \text{ or } 37.5\%$$

Relativni rizik i relativno smanjenje rizika

U usporedbi s ARR, relativni rizik (engl. *relative risk*, RR) podrazumijeva sasvim drugačiji pogled na rizik. On iskazuje rizik od bolesti kod liječene skupine *u odnosu* na rizik kod neliječene skupine. Budući da se radi o testu određivanja relativnog rizika, on se ne može baš tako jasno i jednostavno objasniti kao test određivanja apsolutnog smanjenja rizika. Međutim, određivanje relativnog rizika nam pruža drugačiju perspektivu vrijednosti liječenja u odnosu na neliječenje.
RR se računa na sljedeći način:

$$\mathbf{RR = \frac{Rizik\ kod\ liječene\ populacije}{Rizik\ kod\ neliječene\ populacije.}}$$

Recimo da će 31,6% starijih odraslih osoba u nekoj zajednici koji ne primaju cjepivo protiv gripe dobiti gripu. Recimo nadalje da će ih 16,4% dobiti gripu iako su primili cjepivo. Kao što znamo ARR kod cijepljenih osoba je 15,2%. Drugi način da se opiše ova situacija jest da će 68,4% odraslih ostati zdravi bez cijepljenja, dok će 83,6% ostati zdravi ako se cijepe.
Relativni rizik se računa na sljedeći način:

$$\mathbf{RR = \frac{Rizik\ (vjerojatnost)\ dobivanja\ gripe\ bez\ cijepljenja}{Rizik\ (vjerojatnost)\ dobivanja\ gripe\ sa\ cijepljenjem} =}$$

$$\mathbf{= \frac{0{,}316}{0{,}164} = 1{,}93}$$

Tumači se na sjedeći način: Rizik dobivanja gripe bez cijepljenja je 1,93 pomnoženo s rizikom od dobivanja gripe usprkos cijepljenju (4). No, to nije isto kao i postotak smanjenja rizika koje pruža cijepljenje. Statistički test koji daje podatak o smanjenju rizika u odnosu na činjenicu je li netko cijepljen ili nije zove se relativno smanjenje rizika (engl. *relative risk reduction*, RRR).
Relativno smanjenje rizika je smanjenje rizika (u postotku) događaja kod liječenih osoba. RRR se računa sljedećom formulom:

$$\mathbf{RRR = \frac{ARR}{Osnovni\ rizik} = RRR\ (1).}$$

RRR od dobivanja gripe kod bolesnika cijepljenih protiv gripe je stoga:

$$\mathrm{RRR} = \frac{15{,}2\%}{31{,}6\%} = 48{,}1\%$$

RRR izražava u kojoj mjeri cjepivo smanjuje rizik od gripe *u odnosu* na prirodni rizik odnosno rizik kod osoba koje se nisu cijepile. Može se tumačiti na sljedeći način: cijepljenjem će smanjiti rizik kod starijih osoba od gripe za 48,1% *u odnosu* na rizik kojem se izlažu u slučaju da se ne cijepe.

Relative risk and relative risk reduction

Relative risk (RR) is a very different way of looking at risk as compared to ARR. It examines the risk of the disease in the treated group *relative* to the risk in the untreated group. Because it is a relative risk statistic, it is not as clearly explained as absolute risk reduction. However, it provides a different perspective on the value of the treatment relative to no treatment.
RR is calculated as follows:

$$\mathbf{RR = \frac{Risk\ in\ treated\ population}{Risk\ in\ untreated\ population.}}$$

Let us assume that 31.6% of elderly adults in the community who do not receive the influenza vaccine will contract the disease. Let us further assume that 16.4% will contract the disease even after having received the vaccine. As we know, the ARR from the vaccine is 15.2%. Another way to describe this situation is that 68.4% of adults will remain healthy without the vaccine while 83.6% will remain healthy if they receive the vaccine.
The relative risk is calculated as follows:

$$\mathbf{RR = \frac{Risk\ (probability)\ of\ contracting\ influenza\ without\ the\ vaccine}{Risk\ (probability)\ of\ contracting\ influenza\ with\ the\ vaccine}}$$

$$\mathbf{= \frac{0.316}{0.164} = 1.93}$$

The way this is interpreted is as follows: The risk of contracting influenza without the vaccine is 1.93 times the risk of contracting influenza with the vaccine (4). But this is not the same thing as the percent reduction in risk that the vaccine provides. The statistic that provides information on reduction of risk relative to having the vaccine is called Relative Risk Reduction (RRR).
Relative risk reduction is the percent reduction in risk of the event that is obtained through treatment. RRR is calculated via the following formula:

$$\mathbf{RRR = \frac{ARR}{Baseline\ risk} = RRR\ (1).}$$

The RRR of contracting influenza for patients who receive the influenza vaccine is therefore:

$$\mathrm{RRR} = \frac{15.2\%}{31.6\%} = 48.1\%$$

The RRR expresses how much the vaccine reduces the risk of influenza disease *relative* to the natural, untreated risk. It can be interpreted as follows: being vaccinated will reduce the elder's risk of influenza by 48.1% *relative* to the risk they incur if they refuse the vaccine.

Jedna od uporaba ovog testa jest pomoć liječniku i bolesniku u odlučivanju je li skupo ili bolno liječenje vrijedno smanjenja rizika koje pruža. Obzirom na činjenicu da cijepljenje protiv gripe stoji samo oko 20 USD, a gripa kod starijih odraslih osoba može vrlo lako rezultirati ozbiljnom bolešću i hospitalizacijom, pa čak i smrću, nije teško donijeti odluku o cijepljenju. Međutim, recimo da određeno liječenje raka bolesniku uzrokuje velike patnje, slabost, povraćanje i druge nuspojave, a njegovo relativno smanjenje rizika iznosi samo 5%. U toj bi se situaciji neki bolesnici odlučili ne započeti s liječenjem.

Na određivanje RRR utječe osnovni rizik (engl. *baseline risk*) što njegovo tumačenje čini kompliciranijim od određivanja ARR. Kako se povećava osnovni rizik, tako raste i RRR, no puno sporije u usporedbi s ARR (opet, pod pretpostavkom da je rizik kod liječenih osoba konstantan) (tablica 2). Jasno je da je kod niskih vrijednosti osnovnog rizika RRR mnogo viši od ARR, no kod viših vrijednosti osnovnog rizika ARR se približava RRR.

Zbog osjetljivosti RRR prema osnovnom riziku važno je da kliničar uzme u obzir oba statistička testa (ARR i RRR) kako bi mogao procijeniti smanjenje rizika do kojeg se može doći uzimanjem određenog lijeka ili početkom određenog liječenja u skupini bolesnika. Također je važno zapamtiti da svaki pojedinačni bolesnik može ili ne mora imati koristi od određene intervencije. Ovi statistički testovi nude procjenu za populaciju, ne za svakog bolesnika ponaosob.

One use of this statistic is to assist physicians and patients to decide if an expensive or painful treatment is worth the reduction in risk it offers. Given that the influenza vaccine costs only about $20.00 and influenza in the elderly can easily result in serious illness and hospitalization, or even death, the decision to be vaccinated is not very difficult. However, suppose a particular cancer treatment caused the patient great suffering from weakness, vomiting and other side-effects, and its relative risk reduction was only 5%. In that situation some patients would forego the treatment.

The RRR statistic is affected by the baseline risk and this fact makes its interpretation more complex than the ARR statistic. As the baseline risk rises, the RRR also increases but at a much lower rate as compared with the ARR (again, assuming the treated risk is constant) (Table 2). It is clear that at lower baseline risk levels, the RRR is much higher than the ARR, but at higher baseline risk levels, the ARR approaches the RRR.

Given the sensitivity of RRR to the baseline risk, it is important for the clinician to consider both the ARR and RRR statistics in order to understand the amount of risk reduction a drug or other treatment can achieve for a population of patients. It is also important to remember that any individual patient may benefit or not benefit from a particular intervention. These statistics predict for populations, not for individuals.

Tablica 2. Učinak osnovnog rizika na relativno smanjene rizika

Table 2. Effect of baseline risk on relative risk reduction

Baseline Risk	Treated Risk	ARR	RRR
48%	31%	17%	35.4%
55%	31%	24%	43.6%
65%	31%	34%	52.3%
75%	31%	44%	58.7%
85%	31%	54%	63.5%
95%	31%	64%	67.4%

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Uloga prolaktina kod raka dojke

The role of prolactin in human breast cancer

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Sažetak

Mnogi su objavljeni podaci o raku dojke i prolaktinu (PRL) proturječni. PRL je prvo bio prepoznat kao hormon koji ima važnu ulogu u inicijaciji i napredovanju raka dojke kod glodavaca i barem djelomično kod ljudi. Ljudske stanice raka dojke u kulturi sintetiziraju biološki aktivni PRL te on djeluje u autokrinoj/parakrinoj stimulacijskoj petlji *(*engl. *autocrine/paracrine stimulatory loop*) unutar tkiva dojke. Aktivnošću tog liganda posreduje izooblik prolaktinskog receptora (receptor prolaktina, PRLR) koji se nalazi na epitelu, odnosno kojeg luči epitel dojke kod ljudi. Kompleks PRL/PRLR se povezuje te aktivira nekoliko signalnih putova koje dijeli s drugim članovima nadskupine receptora citokina. Prijenos signala PRLR započinje s tri tirozin-kinaze, odnosno Jak2, Src i Tec. U nedavno objavljenim podacima upućuje se na funkcionalnu ulogu PRL unutar jezgre gdje on djeluje zajedno s ciklofilinom B kao pobuđivačem transkripcije. Nekoliko epidemioloških istraživanja ukazalo je na moguću funkciju PRL kao čimbenika napredovanja raka dojke kod ljudi. PRL bi mogao biti važan lokalni promotor rasta uključen u patogenezu raka dojke kod žena. Hiperprolaktinemija bi mogla biti pokazateljem napredovanja bolesti i nepovoljne prognoze. U kliničke pristupe kontroliranju bolesti trebalo bi uključiti antagoniste interakcije PRL/PRLR ili signalnu transdukciju povezanu s receptorom PRL.

Ključne riječi: prolaktin; rak dojke; receptori prolaktina; signalni putevi prolaktina; hiperprolaktinemija

Abstract

Much of the literature on human breast cancer and prolactin (PRL) appears to be contradictory. PRL has been first recognized as a hormone that plays an important role in breast cancer initiation and development in rodents, and, at least partly, in humans. Bioactive PRL is synthesized by human breast cancer cells in culture and acts in an autocrine/paracrine stimulatory loop within breast tissue. The actions of this ligand are mediated by PRL receptor (PRLR) isoforms found on, or secreted by, human breast epithelium. The PRL/PRLR complex associates with, and activates, several signaling pathways that are shared with other members of the cytokine receptor superfamily. Proximal PRLR signaling is initiated by three tyrosine kinases, namely Jak2, Src, and Tec. Some recent literature data have indicated a functional role for PRL within the nucleus where it acts in a complex with cyclophilin B as a transcriptional inducer. Several epidemiological studies have indicated that PRL may also function as a progression factor for human breast cancer. PRL might be an important local growth promoter involved in the pathogenesis of human breast cancer. Hyperprolactinemia could be an indicator of disease progression and poor prognosis and clinical approaches to controlling this disease need to incorporate antagonists of PRL/PRLR interaction or PRL receptor-associated signal transduction.

Key words: prolactin; breast cancer; prolactin receptors; prolactin signaling pathways; hyperprolactinemia

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Uvod

Čini se da je sve više podataka o ulozi prolaktina (PRL) kod raka dojke proturječno; stoga je teško ustanoviti ulogu PRL u raku dojke kod žena. PRL je prvo bio prepoznat kao hormon koji ima važnu ulogu u pokretanju i napredovanju raka dojke kod glodavaca (1,2) te barem djelomično kod žena. Postoji i čvrst dokaz izravne stimulirajuće uloge PRL na epitelne stanice tkiva dojke (3,4) i stanice raka doj-

Introduction

Accumulated data about the role of prolactin (PRL) in human breast cancer appear to be controversial. Thus, it has been difficult to establish definitive involvement of PRL in human breast disease. PRL was first recognized as a hormone that plays an important role in breast cancer initiation and development in rodents (1,2) and, at least partly, in humans. There is also firm evidence

ke u kulturi (5,6). Postoje i neka epidemiološka istraživanja koja pokazuju značajno povišenje koncentracije PRL u serumu kod određenih podskupina bolesnica oboljelih od raka dojke (7-10) i kod žena s rizikom od raka dojke zbog povijesti raka dojke u obitelji (9,11). Ovaj članak ispituje ima li osnove za tvrdnju kako PRL ima ulogu koja doprinosi onkogenezi tumora dojke.

Aktivnost PRL u tkivu dojke – istraživanja *in vitro*

Sinteza i izlučivanje PRL iz stanica raka dojke

Danas je već poznato da kod sisavaca postoje endokrini i autokrini/parakrini izvori PRL. Godinama su objavljivani radovi u kojima se nagađalo o mogućim drugim izvorima PRL osim hipofize. Podaci iz 1970. pokazuju da su kod bolesnika s rakom dojke koji su prošli hipofizektomiju koncentracije PRL bile približne normalnim koncentracijama (12,13), dok su imunohistokemijska istraživanja otkrila izražaj ekspresiju imunoreaktivnog proteina PRL u epitelu dojke (14). Uz to je niska koncentracija cirkulirajućeg PRL prevladala kod bolesnika na terapiji kojom se potiskuje djelovanje hormona hipofize (engl. *pituitary hormone suppression therapy*) (15). Istraživanja iz ranih 1990. ukazala su da mRNA za PRL može biti prisutna u normalnom i neoplastičnom epitelu dojke kod žena (16–18) i epitelu tkiva dojke kod skotnih ženki glodavaca (19,20). PRL stvaraju tumori kao i razna zdrava tkiva. Posteljica je najbogatiji izvor PRL kojeg ne luči hipofiza (21) i odgovorna je za njegovu visoku koncentraciju u plodnoj vodi kod žena. Imunološki sustav, maternica, te moždani i kožni fibroblasti također proizvode PRL (21–24). Ti su rezultati doveli laboratorije Vonderhaar i Clevenger (17,18) do postavljanja hipoteze i naposljetku do dokaza da se PRL sintetizira i izlučuje u tkivu i stanicama dojke kod žena. Biološki aktivan PRL sintetiziraju stanice raka dojke u kulturi te on djeluje kao autokrina/parakrina stimulacijska petlja u tkivu dojke, što ukazuje na ulogu ovog hormona u patogenezi raka dojke (17,18). Rast dviju vrsti stanica raka dojke kod žena, tj. T47Dco (negativne na estrogenski receptor, ER-negativno) i MCF–7 (pozitivne na estrogenski receptor, ER-pozitivno), spriječen je nakon liječenja monoklonskim protutijelima usmjerenima na ljudski PRL iz hipofize (17). Uz to, protusmislena (engl. *antisense*) RNA usmjerena na gen za PRL iz hipofize znatno je pojačala rast stanica T47Dco (25). Prisutnost mRNA za PRL u stanicama T47Dco i MCF-7 potvrđena je metodom lančane reakcije polimerazom nakon reverzne transkripcije (obrnutog prepisivanja) (engl. *reverse transcription polymerase chain reaction*, RT-PRC) (17) te je mRNA za PRL bila prisutna kod 82% ispitanih linija stanica raka dojke (25). Nadalje, velika većina, odnosno 98% raka dojke kod žena sintetizira PRL mRNA, kao što je otkriveno hibridizacijom *in situ* (26). Neka is-

of a direct stimulatory role of PRL on mammary epithelial cells (3,4), and breast cancer cells in culture (5,6). There are also some epidemiological studies showing significant increase in serum PRL concentrations in certain subpopulations of breast cancer patients (7-10), and in women at risk of developing familial breast cancer (9,11). This paper examines the basis for a claim that PRL has a contributory role during breast oncogenesis.

Actions of PRL within mammary tissues – *in vitro* investigations

Synthesis and secretion of PRL by breast cancer cells

Both endocrine and autocrine/paracrine sources of PRL have been recognized to exist in mammals. For many years, evidence in the literature has hinted at the possibility of an extrapituitary source of PRL. Evidence from the 1970's indicated that hypophysectomized breast cancer patients had near-normal PRL levels (12,13), whereas immunohistochemistry studies revealed the expression of immunoreactive PRL protein in human breast epithelium (14). In addition, low levels of circulating PRL were found to persist in patients under pituitary hormone suppression therapy (15). Studies in the early 1990's indicated that the mRNA for PRL could be found in normal and neoplastic human breast epithelium (16-18), and mammary epithelium from pregnant rodents (19,20). PRL is generated by tumors as well as by a variety of normal tissues. Placenta is the richest source of the extrapituitary PRL (21), and is responsible for its high level in human amniotic fluid. Immune system, uterus, brain and dermal fibroblasts also produce PRL (21-24). These findings led both Vonderhaar and Clevenger laboratories (17,18) to hypothesize and subsequently prove that PRL is synthesized and secreted in human breast tissues and cells. Bioactive PRL is synthesized by human breast cancer cells in culture and acts in an autocrine/paracrine stimulatory loop within breast tissue, suggesting a role for this hormone in the pathogenesis of breast cancer (17,18). The growth of both T47Dco (ER-negative) and MCF-7 (ER-positive) human breast cancer cells was inhibited after treatment with monoclonal antibodies raised against human pituitary PRL (17). In addition, antisense RNA directed against the gene encoding for pituitary PRL significantly inhibited growth of T47Dco cells (25). The presence of the mRNA for PRL in T47Dco and MCF-7 was confirmed by RT-PCR (reverse transcription polymerase chain reaction) (17), and 82% of all tested breast cancer cell lines contained mRNA for PRL (25). Furthermore, the vast majority, i.e. 98% of human breast cancers synthesize PRL mRNA as detected by *in situ* hybridization (26). In addition, some studies revealed that more than 75% of primary breast cancer surgical samples also contain mRNA for PRL, and, in the majority

traživanja otkrila su da je mRNA za PRL također bila prisutna kod više od 75% primarnih kirurških uzoraka raka dojke te da je u većini slučajeva koncentracija mRNA za PRL i njegove receptore bila značajno povišena kod malignih tkiva u odnosu na okolna, nemaligna tkiva kod istog bolesnika (16,25,27).

Receptori prolaktina (PRLR)

Aktivnost PRL u mliječnoj žlijezdi zahtijeva prisutnost površinskog receptora njegove srodne stanice, PRLR. PRLR pripada citokinskoj hematopoetskoj obitelji receptora (28,29). Članovi te nadskupine su jednomembranski receptori s tri domene: domenom koja veže izvanstanični ligand, hidrofobnom transmembranskom domenom i unutarstaničnom domenom bogatom prolinom. Postoje barem tri različita izooblika PRLR koji se uglavnom razlikuju po svojoj citoplazmatskoj domeni: dugi (90 kDa), srednji i kratki (40 kDa). Dugi i kratki izooblici PRLR stvoreni su alternativnim cijepanjem mRNA jednog gena i razlikuju se samo po duljini citoplazmatske domene (30). Srednji oblik je delecijski mutant dugog oblika kojem nedostaje 198 aminokiselina u svojoj citoplazmatskoj domeni. Najkraći izooblik PRLR, PRL-vezni protein (engl. *PRL binding protein*, PRLBP) identificiran je u ljudskom serumu (31) i predstavlja slobodno cirkulirajuću izvanstaničnu domenu PRLR (32). Sva tri izooblika pospješuju mitozu (33). I zdrave i maligne stanice raka dojke sadržavaju kratke i duge oblike PRLR (34,35), dok je srednji oblik nađen kod stanica Nb2 limfoma te je osjetljiviji na PRL u usporedbi s druga dva oblika PRLR (36). Srednji oblik PRLR bio je otkriven u uzorcima tkiva dojke (37), što je suprotno rezultatima nekih drugih istraživanja (18). Prednosti imunohistokemije, hibridizacije *in situ* i RT-PCR jesu da omogućuju bolju procjenu koncentracije PRLR kod raka dojke kod žena, te su rezultati nekih istraživanja provedenih tim metodama (26,35,37,38) otkrila da je hPRLR izražen kod 98% svih slučajeva raka dojke kod žena. Istraživanja koja istražuju izražaj PRLR na razini mRNA ukazuju na vezu ili s izražajem ER/PR (estrogenskim/progesteronskim receptorima) (38) ili neoplazijom (37); međutim, istraživanja na razini proteina nisu potvrdila ta promatranja (26). PRLR se obično stabilizira kod raka dojke zbog smanjene fosforilacije ostatka Ser349 koji, kada je fosforiliziran, koristi beta Trcp E3 ubikvitin-ligazu te pomaže pri razgradnji PRLR (39).

Neoplastični razvoj i napredovanje bolesti zahtijevaju dereguliranu proliferaciju stanice, povećano preživljavanje stanica, dobivanje odgovarajuće vaskularne opskrbe i neograničavanje pokretnosti. Iako postoje dokazi da PRL može inicirati rast i pokretnost stanica raka dojke kod žena, njegova nesposobnost kod iniciranja diferencijacije i dalje ostaje nerazjašnjena. Potencijalni mehanizmi za to uključuju promjene u koncentraciji Stat5 (prenositelja signala i aktivatora transkripcije) (engl. *signal transducer and activator of transcription*, STAT) ili fosforilaciji, kvan-

of cases, the amount of mRNA for PRL and its receptors is significantly elevated in malignant vs. the adjacent, non-malignant tissue from the same patient (16,25,27).

PRL receptors (PRLR)

The actions of PRL in the mammary gland require the presence of its cognate cell surface receptor, the PRLR. PRLR belongs to the cytokine hematopoietic family of receptors (28,29). The members of this superfamily are single membrane-spanning receptors with three domains: an extracellular ligand binding domain, a hydrophobic transmembrane domain, and an intracellular proline-rich domain. There are at least three different isoforms of PRLR differing mainly in their cytoplasmic domain: long (90 kDa), intermediate, and short (40kDa) isoform. Long and short isoforms of PRLR are generated by differential splicing of a single gene and differ only in the length of the cytoplasmic domain (30). The intermediate form is a deletion mutant of the long form, lacking 198 aminoacids in its cytoplasmic domain. The shortest PRLR isoform, the PRL binding protein (PRLBP) was identified in human serum (31) and it represents the freely circulating extracellular domain of the PRLR (32). All three isoforms promote mitosis (33). Both normal and malignant mammary cells contain both the long and short forms of PRLR (34,35), while the intermediate form is found in Nb2 lymphoma cells and is more sensitive to PRL compared with the other two forms of PRLR (36). The intermediate form of PRLR has been detected in breast tissue samples (37), in contrast to the results from another study (18). Advances in immunohistochemistry, *in situ* hybridization, and RT-PCR enabled increasingly sensitive estimation of the PRLR in human breast cancer, and results of some of the studies using these technologies (26,35,37,38) have revealed that the hPRLR is expressed in up to 98% of all human breast cancers. The studies examining PRLR expression at the mRNA level have suggested an association with either ER/PR (estrogen/progesterone receptors) expression (38) or neoplasia (37); however, studies at the protein level have not confirmed these observations (26). The PRLR are commonly stabilized in human breast cancer due to decreased phosphorylation of residue Ser349 which, when phosphorylated, recruits the beta Trcp E3 ubiquitin ligase and facilitates PRLR degradation (39).

Neoplastic development and progression require deregulated cell proliferation, increased cellular survival, acquisition of an adequate vascular supply, and escape from constraints on motility. Despite evidence that PRL can trigger the growth and motility of human breast cancer cells, the inability of PRL to trigger differentiation remains uncertain. Potential mechanisms include alterations in Stat5 (signal transducer and activator of transcription) levels or phosphorylation, quantitative changes in the expression of various hPRLR isoforms, or alteration in

titativne promjene u ekspresiji raznih izooblika hPRLR ili promjeni reakcije malignih epitelnih stanica na bazalnu membranu, što bi moglo izravno utjecati na prijenos signala PRLR (32). Budući da je poboljšana pokretnost jedan od aspekata metastatskog procesa, postavlja se pitanje može li PRL služiti kao kemoatraktant za rak dojke kod žena *in vitro* (40). PRL stimulira citoskeletnu reorganizaciju i pokretnost stanica raka dojke. Tijekom prijenosa signala PRLR Vav2 (čimbenik izmjene gvanin-nukleotida) (engl. *guanine nucleotide exchange factor,* GEF) postaje fosforiliziran i aktivira se, što regulira serin/treonin-kinaza Nek3 (engl. *never in mitosis gene a-related kinase 3*), a što pak doprinosi širenju raka dojke preko PRL mehanizama koji uključuju aktivaciju Rac1 (čimbenik izmjene gvanin-nukleotida) i fosforilaciju paksilina (41).

Također je moguće da PRL utječe na karcinogenezu raka dojke na način da modulira vaskularizaciju. Pokazano je da sam hPRL, isto kao i ljudski GH (engl. *growth hormone,* GH) i placentalni hormoni, može stimulirati stvaranje kapilara kod CAM-testa (engl. *chicken chorioallantoic membrane assay,* CAM) (42). Suprotno tome, produkt proteolitičkog cijepanja PRL, 16K-PRL, jest snažan antiangiogenski agens *in vivo* i *in vitro* (42-44). Taj je N-terminalni produkt cijepanja PRL spriječio proliferaciju endotelnih stanica kao odgovor na čimbenik rasta endotelnih stanica (engl. *vascular endothelial growth factor*, VEGF) i čimbenik rasta osnovnog fibroblasta (engl. *basic fibroblast growth factor*, BFGF) na način da je zapriječio signalni put Ras-Raf1-MAPK i pojačao ekspresiju inhibitora aktivacije plazminogena tipa 1 (engl. *type 1 plasminogen activator inhibitor*) (42,45,46). Čini se da tim aktivnostima posreduje neki receptor koji nije PRL (47). Indukcija VEGF prolaktinom bila je ovisna o PRLR, Jak2 i MAP-kinazi. PRL potiče ekspresiju VEGF preko Erg-1 (engl. *ether-a-go-go-related gene 1 encoded K^+ channels*) i koristi VEGF kao posrednika angiogeneze koju regulira PRL (48).

Uloga PRL kod raka dojke je kompliciranа, čemu posebno pridonosi činjenica da je sam PRL angiogeničan, a proteaze cijepaju PRL kako bi stvorile vazoinhibine (engl. *vasoinhibins*), obitelj peptida koji djeluju na endotelnim stanicama kako bi potisnuli angiogenezu i vazodilaciju te potakli vaskularnu regresiju kojoj posreduje apoptoza (49).

Signalni putovi PRL/PRLR

Nakon dimerizacije receptora potaknute PRL, aktivira se nekoliko različitih kinaza kako bi prenijele hormonski signal. Prijenos signala PRLR iniciraju tri tirozin-kinaze: Jak2 (Janus-kinaza), Src (sarkoma) i Tec (proteinska tirozin-kinaza) (32). Te kinaze pripadaju Janus-obitelji kinaza (Jak skupina). U signalni put PRL uključeni su i višestruki dodatni nizvodni putevi kao što su Src-obitelj kinaza, Ras-MAPKs (štakorske sarkoma-mitogenski aktivirane proteinske kinaze) i PI3K (fosfoinozitid-tri-kinaza) (50) (Slika 1).

the malignant epithelial cell's responsiveness to the basement membrane, which could indirectly impact PRLR signaling (32). Because enhanced motility is one aspect of the metastatic process, it has been questioned whether PRL could serve as a chemoattractant for human breast cancer *in vitro* (40). PRL stimulates the cytoskeletal r-organization and motility of breast cancer cells. During PRLR signaling, Vav2 (guanine nucleotide exchange factor) becomes phosphorylated and activated, an event regulated by the serine/threonine kinase Nek3 (never in mitosis gene a-related kinase 3) which contributes to PRL-mediated breast cancer motility through mechanisms involving Rac1 (guanine nucleotide exchange factor) activation and paxillin phosphorylation (41).

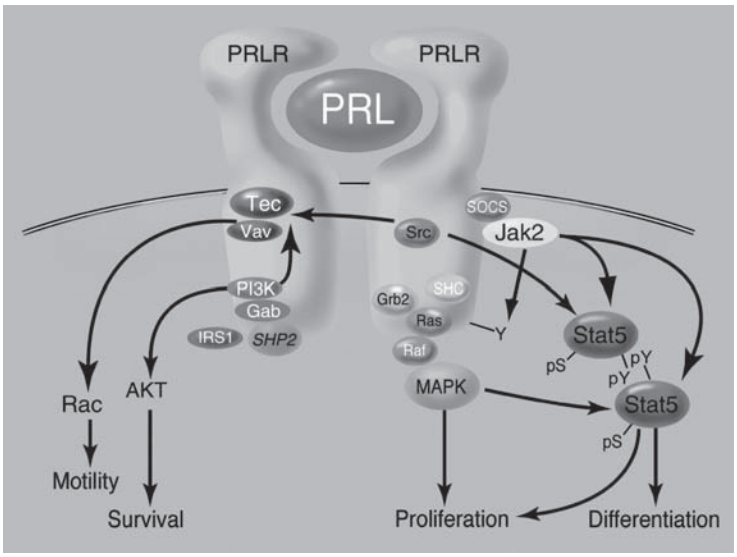
PRL may also influence mammary carcinogenesis by modulating vascularization. It was shown that hPRL itself, as well as human GH, and placental hormones, could also stimulate formation of capillaries in the chicken chorioallantoic membrane assay (42). In contrast, a proteolytic cleavage product of PRL, 16K-PRL, is a potent antiangiogenic agent *in vivo* and *in vitro* (42-44). This N-terminal cleavage product of PRL inhibited endothelial cell proliferation in response to vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (BFGF) by inhibiting the Ras-Raf1-MAPK signaling pathway and increasing expression of type 1 plasminogen activator inhibitor (42,45,46). These activities appear to be mediated by a receptor distinct from the PRLR (47). The induction of VEGF by PRL is PRLR-, Jak2-, and MAP kinase-dependent. PRL induces VEGF expression through Erg-1 (ether-a-go-go-related gene 1 encoded K^+ channels), and implicates VEGF as an intermediary of PRL-regulated angiogenesis (48).

The role of PRL in breast cancer is complicated by the fact that PRL itself is angiogenic, but proteases cleave PRL to generate vasoinhibins, a family of peptides that act on endothelial cells to suppress angiogenesis and vasodilation and to promote apoptosis-mediated vascular regression (49).

PRL/PRLR signaling pathways

Upon PRL-induced receptor dimerization, several different kinases are activated to transduce the hormonal signal. Proximal PRLR signaling is initiated by three tyrosine kinases, namely Jak2 (Janus kinase), Src (sarcoma), and Tec (protein tyrosine kinase) (32). These kinases belong to the Janus family of kinases (Jak family). Multiple additional downstream pathways, such as Src family kinases, Ras-MAPKs (rat sacoma-mitogen-activated protein kinases), and PI3K (phosphoinositide 3 kinase) are involved in PRL signaling (50) (Figure 1).

Although it is not clear that all PRL signaling requires Jak2 as a proximal intermediate (50,51), a great deal of evidence in many cell types supports a key role for this ki-



Slika 1. Aspekti signalnih puteva receptora prolaktina (PRLR). Dimerizacija potaknuta receptorom PRL pokreće povezanost s Jak2-kinazom te rezultira aktivacijom Jak2, fosforilacijom PRLR, te vezanjem i fosforilacijom proteina Stat5. Prijenos signala kroz put SHC/GRB2/Ras/Raf/MEK/MAPK također izravno potiče proliferaciju i modulira aktivnost Stat-proteina. Kompleks između Tec tirozin-kinaze i Vav obitelji čimbenika izmjene gvanin-nukleotida također je povezan s PRLR vezanim za ligand, što rezultira njegovom aktivnošću i stimulacijom stanične pokretnosti (modificirano prema popisu literature, br. 32).

Figure 1. Aspects of PRLR signaling pathways. PRL-induced receptor dimerization induces the association of the Jak2 kinase, resulting in the activation of Jak2, PRLR phosphorylation, and the association and phosphorylation of Stat5. Signaling through the SHC/GRB2/Ras/Raf/MEK/MAPK pathway also directly stimulates proliferation and modulates Stat activity. The complex between the Tec tyrosine kinase and the Vav family of guanine nucleotide exchange factors also inducibly associates with ligand-bound PRLR resulting in its activation and stimulation of cellular motility (modified according to ref. 32).

Iako nije do kraja razjašnjeno da svi signalni putovi PRL zahtijevaju Jak2 kao bliskog posrednika (50,51), mnogo dokaza iz različitih tipova stanica podržava ključnu ulogu te kinaze kod mnogih aktivnosti PRL (52,53). Jak2 fosforilira višestruke supstrate uključujući PRLR i sam Jak2. Time se osiguravaju mjesta molekularnog modeliranja (engl. *docking sites*) proteina s domenom SH2 (engl. *src homology2*, SH2) uključujući i Stat-proteine. Vezanje PRL za svoje receptore vodi ka tirozinskoj fosforilaciji citoplazmatskih transkripcijskih čimbenika, uglavnom članova obitelji Stat (32,54). Aktivacija Stat-proteina rezultira njihovom translokacijom u jezgri, nakon čega slijedi aktivacija prepisivanja transkripcije gena (32,55). Stat1, Stat3 i Stat5 aktiviraju se u stanicama T47D nakon 15-minutne izloženosti PRL (56). Nekoliko je istraživanja pokazalo povećanje koncentracije proteina Stat1 i 3 kod primarnih tumora dojke (57,58). Međutim, njihovi ciljni geni u procesu onkogeneze, relativna važnost PRL u njihovoj regulaciji te razlike u odnosu na zdrave mliječne žlijezde nisu još objašnjeni u dovoljnoj mjeri. Proteini Stat 3 i 5 sudjeluju u prolaktinskoj aktivaciji promotora ciklina D1 (59) te ukazuju na barem jedan cilj PRL na tom putu koji bi mogao doprinijeti stvaranju tumora. Pokazano je (60) da je Stat5b, a ne Stat5a, mogući posrednik stvaranja tumora pobuđenog sa Src. U skladu s prethodno iznesenim, fosforilacija tirozina Jak2-kinazom povezanom s receptorom rezultira dimerizacijom/multi-

nase in many actions of PRL (52,53). Jak2 phosphorylates multiple substrates, including the PRLR and Jak2 themselves. This provides docking sites for proteins with SH2 (src homology2) domains, including Stats. The binding of PRL to its receptors induces tyrosine phosphorylation of cytoplasmic transcription factors, mainly Stat family members (32,54). Activation of Stat proteins results in their translocation to the nucleus, and subsequent activation of gene transcription (32,55). Stat1, Stat3 and Stat5 are activated in T47D cells within 15 min of PRL treatment (56). Several studies have demonstrated increased levels of Stats1 and 3 in primary mammary tumors (57,58). However, their target genes in oncogenic processes, the relative importance of PRL in their regulation, and differences from the normal mammary gland have not been understood. Both Stats3 and 5 are involved in PRL activation of the cyclin D1 promoter (59), suggesting at least one target of PRL through this pathway that could contribute to tumorigenesis. It has been demonstrated (60) that Stat5b, rather than Stat5a, is a potent mediator of Src-induced tumorigenesis. As discussed above, tyrosine phosphorylation by a receptor-associated Jak2 kinase results in the dimerization/multimerization and nuclear retrotranslocation of the Stat complex where it engages its cognate DNA binding sequence, resulting in promoter transactivation under appropriate conditions (32,61).

merizacijom i nuklearnom retrotranslokacijom kompleksa Stat uz zahvaćanje njegove srodne vezne sekvence DNA, što rezultira transaktivacijom promotora pod određenim uvjetima (32,61). Dok je PRL stimulirao tirozinsku fosforilaciju kod Stat 5a i 5b, aktivacija Src je rezultirala tirozinskom fosforilacijom Stat 5a i 5b i nuklearnom translokacijom, ali samo Stat 5b (62). Pokazano je da PRL aktivira Src u nizu različitih tipova stanica uključujući i stanice jetre štakora (63). Rezultati nekih nedavno provedenih istraživanja ukazuju da protoonkogen c-Myc, koji možda funkcionira kao koaktivator Stat 5a, pojačava izražaj gena koji uzrokuje Stat5a, kod raka dojke kod žena (54,64). Nadalje, PRL stimulira ubikvitinaciju, internalizaciju i razgradnju njegovih receptora pomoću katalitičke aktivacije Jak2 (65) te se čini da je fosforilacija PRLR pomoću Ser349 ključni događaj u tom složenom provođenju signala kojem posreduje Jak2.

Signalni put Ras-Raf-MAPK

Signalni put Ras-Raf-MAPK je mehanizam koji posreduje kod proliferacijske aktivnosti čitavog niza čimbenika rasta i citokina. Raf1 (mitogenski aktivirana proteinska kinaza-kinaza-kinaza), MEK (mitogenski aktivirana proteinska kinaza-kinaza) i MAP (mitogenski aktivirana proteinska kinaza) su nizvodne kinaze na tom signalnom putu. Prijenos signala pomoću puta SHC/GRB2/Ras/Raf/MEK/MAPK (SHC-adapterski protein; GRB2-receptor faktora rasta-vezni protein2; Ras-štakorski sarkom) također izravno potiču proliferaciju i moduliraju aktivnost Stat. Pokazano je da PRL aktivira taj put u velikom broju modela ovisnim o PRL (66) i staničnim linijama raka dojke (67-69) kao i kod zdravih mišjih epitelnih stanica dojke (68,69). PRL također može sinergijski aktivirati taj put preko komunikacije s drugim čimbenicima rasta ovisno o fenotipu tumorskih stanica. Aktivacija Jak2 koju pobuđuje PRL rezultirala je tirozinskom fosforilacijom erbB2, pritom povećavajući povezanost s Grb2 i aktivirajući put Ras-MAPK (70) te uključujući fosforilaciju nekih transkripcijskih čimbenika i na taj način povećavajući sintezu produkata gena obitelji fos. O komunikaciji između putova Stat i MAPK na drugim točkama objavljeno je dovoljno podataka za mnoge citokine, uključujući i PRL (71). MAPK mogu fosforilizirati Stat-proteine na serinskim i treoninskim ostacima, čime se pojačavaju aktivnosti proteina Stat 1 i 3 (72).

Putevi PI3K (fosfoinozitid-3-kinaza)

Kompleks između Tec tirozin-kinaze i Vav obitelji čimbenika izmjene gvanin-nukleotida također se inducirano povezuje s PRLR vezanim za ligand. To rezultira izmjenom GDP i GTP na malom G-proteinu Ras, što dovodi do njegove aktivacije i poticanja stanične pokretnosti. Aktivacija tirozin-kinaze Tec i Akt (PKB - proteinska kinaza B) izravno je vezana za aktivaciju PI3K koju izaziva PRL. SHP-2-fosfataza je također povezana s PRLR te pojačava njegovu ak-

Whereas PRL stimulated tyrosine phosphorylation and nuclear translocation of both Stats5a and 5b, Src activation resulted in tyrosine phosphorylation of both Stats5a and 5b, but nuclear translocation of only Stat5b (62). PRL has been shown to activate Src in a variety of cell types, including rat liver (63). Results from some recent studies indicate that proto-oncogene c-Myc potentiates Stat5a-driven gene expression, possibly functioning as a Stat5a coactivator in human breast cancer (54,64). Furthermore, PRL stimulates ubiquitination, internalization, and degradation of its receptors via catalytic activation of Jak2 (65), and it seems that Ser349 phosphorylation of PRLR is essential event in this complex Jak2-mediated signaling.

Ras-Raf-MAPK pathway

Ras-Raf-MAPK signaling pathway is a mechanism by which a variety of growth factors and cytokines mediate their proliferation action. Raf1 (mitogen activated protein kinase kinase kinase), MEK (mitogen activated protein kinase kinase), and MAP (mitogen activated protein kinase) are downstream kinases in this signaling pathway. Signaling through the SHC/GRB2/Ras/Raf/MEK/MAPK pathway (SHC-adaptor protein; GRB2-growth factor receptor-binding protein2; Ras-rat sarcoma) also directly stimulates proliferation and modulates Stat activity. PRL has been shown to activate this pathway in a number of PRL-dependent models (66), and mammary tumor cell lines (67-69), as well as normal mouse mammary epithelial cells (68,69). PRL can also synergistically activate this pathway via cross-talk with other growth factors, depending on the phenotype of the tumor cell. PRL-induced activation of Jak2 resulted in tyrosine phosphorylation of erbB2, thereby increasing association with Grb2, and activating the Ras-MAPK pathway (70) including phosphorylation of some transcription factors and thus increasing synthesis of the products of the fos gene family. Cross-talk between the Stat and MAPK pathways at other points has been well documented for many cytokines, including PRL (71). MAPKs are able to phosphorylate Stats on serine and threonine residues, which augments the activities of Stats1 and 3 (72).

PI3K (phosphoinositide 3 kinase) pathways

The complex between the Tec tyrosine kinase and the Vav family of guanine nucleotide exchange factors also inducibly associates with ligand-bound PRLR. This leads to the exchange of GDP for GTP on the small G protein Ras, resulting in its activation and stimulation of cellular motility. Activation of tyrosine kinases Tec and Akt (PKB-protein kinase B) is directly linked to the PRL-induced activation of PI3K. The phosphatase SHP-2 also binds to PRLR and potentiates its activity. Activation of PI3K generates phosphoinositides that serve as second messengers and can regulate multiple pathways important in onco-

tivnost. Aktivacija PI3K stvara fosfoinozitide koji služe kao sekundarni glasnici te mogu regulirati višestruke puteve važne u onkogenezi, uključujući proliferaciju i citoskeletnu preraspodjelu, kao i sprječavanje apoptoze i angiogeneze (73-75). Aktivacija ciklusa fosfoinozitida rezultira stimulacijom puta PKC (proteinske kinaze C). Moguće je da bi PRL mogao aktivirati PI3K preko višestrukih dodatnih puteva. PI3K bi mogla biti ciljna destinacija za Ras (76), a pokazano je da se regulacijska podjedinica p85 povezuje s nekoliko nizvodnih efektora i adaptora citokina i receptora čimbenika rasta uključujući Stat5, Stat3, IRS1 (inzulinski receptor-supstrat 1), Gab1 (vezujući protein 1 povezan s GRB2) i Gab2 (vezujući protein 2 povezan s GRB2), te SHP-2 (Src homologija-2 domena koja sadrži protein-tirozin-fosfatazu) (77,78). Fosfoinozitidi koje stvara PI3K stvaraju mjesta molekularnog modeliranja za Akt kao i njegove uzvodne kinaze. Metaboliti fosfoinozitida se možda također vežu za čimbenike izmjene gvanin-nukleotida uključujući i Vav, kao i za Tec, člana veće obitelji tirozin-kinaze. Konstitutivni kompleks Tec i Vav (79) povezuje se s PRLR u stanica raka T47D koje stimulira ligand (80).

Aktivnost PRL unutar jezgre

Neki podaci iz literature ukazuju na funkcionalnu ulogu PRL unutar jezgre (81,82). Ti su podaci proturječni klasičnoj teoriji koja tvrdi da aktivnošću peptidnih hormona posreduju samo receptori na površini stanice. Međutim, podaci nedavno provedenih istraživanja otkrili su ulogu peptidil-prolil-izomeraze ciklofilina B (CypB) u nuklearnom transportu i funkciji PRL (82). Kompleks između PRL i CypB postoji u ljudskom serumu, veže se za PRLR i endocitoziran je tijekom receptorske internalizacije. Unutar jezgre kompleks PRL/CypB djeluje kao transkripcijski pobuđivač na način da uključuje interakciju Stat5 s DNA tako da pokreće oslobađanje represora Stat5, odnosno PIAS3 (83).

Aktivnost PRL kod modela glodavaca

Uloga prolaktina kod raka dojke u glodavaca potkrijepljena je dokumentiranim podacima (1,84). Postoji izravna korelacija između hiperprolaktinemije uzrokovane lijekovima i pojačanog rasta tumora te hipoprolaktinemije i usporenog rasta tumora (1,85). Izloženost PRL poboljšava razvoj kemijski uzrokovanog raka dojke kod glodavaca (1,86,87). Prevelika ekspresija PRL kod transgenskih miševa s povećanom aktivacijom PRLR dovoljna je da uzrokuje stvaranje raka dojke kod starosti od 11-15 mjeseci (88,89). Izloženost estrogenu sa sekundarnim povećanjem koncentracije cirkulirajućeg PRL može prouzročiti ponovnu podložnost kemijskim kancerogenim tvarima kod već ranije skotnih ženki miševa (90). Možemo zaključiti da brojna istraživanja pokazuju ulogu PRL u pojačavanju prihvaćanja kemijskih kancerogenih tvari kod mliječnih žlijezda glodavaca.

genesis, including proliferation and cytoskeletal rearrangements, as well as inhibition of apoptosis and angiogenesis (73-75). Activation of phosphoinositide cycle results in the stimulation of PKC (protein kinase C) pathway. PI3K could potentially be activated by PRL through multiple additional pathways. It can be a target of Ras (76), and the p85 regulatory subunit has been shown to associate with several downstream effectors and adaptors of cytokine and growth factor receptors, including Stat5, Stat3, IRS1 (insulin receptor substrate 1), Gab1 (GRB2-associated binding protein 1) and Gab2 (GRB2-associated binding protein 2), and SHP-2 (Src homology-2 domain-containing protein-tyrosine phosphatase) (77,78). PI3K-generated phosphoinositides provide docking sites for Akt, as well as its upstream kinases. Phosphoinositide metabolites may also bind to guanine nucleotide exchange factors, including Vav, as well as to Tec, a member of a larger family of tyrosine kinases. A constitutive complex of Tec and Vav (79) associates with PRLR in ligand-stimulated T47D (80).

Intranuclear action of PRL

Some literature data have indicated a functional role for PRL within the nucleus (81,82). These data stand in contrast with the classic theory that postulates that peptide hormone action is mediated only by cell surface receptors. Recent data, however, have revealed a role for the peptidyl prolyl isomerase cyclophilin B (CypB) in the nuclear transport and function of PRL (82). A complex between PRL and CypB is found in human serum, binds to the PRLR, and is endocytosed during receptor internalization. The PRL/CypB complex acts within the nucleus as a transcriptional inducer by facilitating the interaction of Stat5 with DNA by inducing the release of a repressor of Stat5, namely PIAS3 (83).

Rodent models of PRL action

Prolactin's role in rodent mammary cancer has been well documented (1,84). There is a direct correlation between drug-induced hyperprolactinemia and increased tumor growth, and hypoprolactinemia and retarded tumor growth (1,85). PRL exposure enhances the development of chemically induced mammary cancers in rodents (1,86,87). Overexpression of PRL in transgenic mice with increased activation of the PRLR is sufficient to induce the formation of mammary cancers at 11–15 months of age (88,89). Exposure to estrogen with secondary increases in circulating PRL levels is able to restore susceptibility to chemical carcinogens in parous mice (90). In summary, numerous studies point to a role for PRL in increasing receptiveness to chemical carcinogens in rodent mammary glands.

Rak dojke kod žena i PRL – istraživanja *in vivo*

Epidemiologija PRL i rak dojke kod žena

Nekoliko epidemioloških istraživanja upućuju na to da PRL može također funkcionirati kao progresijski čimbenik raka dojke kod žena (8,91-93). U velikom se broju istraživanja proučavala povezanost između koncentracije PRL i nekoliko čvrsto potvrđenih čimbenika rizika oboljenja od raka dojke kao što su paritet i dob prilikom prvog poroda, dob kod prve menstruacije i menopauze, povijest raka dojke u obitelji, mamografska gustoća tkiva, etničke razlike, prehrambene navike, uzimanje lijekova i prolaktinomi.

Čini se da se koncentracija PRL snižava sa svakom sljedećom trudnoćom (94,95). Također nije primijećena nezavisna povezanost između dobi kod prvog poroda i koncentracije PRL (94). Nije se izvještavalo niti o značajnoj povezanosti između koncentracije PRL i dobi kod prve menstruacije, odnosno dobi kod nastupa menopauze (94,96). U podskupini s rizikom zbog obiteljske povijesti raka dojke, bazalna koncentracija PRL u serumu bila je statistički značajno povišena (11). Međutim, u nekoliko drugih istraživanja (94-96) jako je malo podataka pronađeno o povezanosti koncentracije PRL s poviješću raka dojke u obitelji.

Više koncentracije PRL primijećene kod žena u menopauzi s većom gustoćom tkiva dojke (94,97) upućuju na mjerljiv utjecaj PRL na epitel dojke i/ili stromalnu proliferaciju.

Nekoliko je istraživanja proučavalo koncentracije PRL i prehrambene navike (95,98), no još se čekaju sukladni rezultati.

Poznato je da velik broj lijekova povećava (oralna kontracepcija, rezerpin, haldol, cimetidin i fenotiazini) ili smanjuje (levodop) koncentraciju PRL u plazmi. Dugoročna primjena oralne kontracepcije povećava rizik od raka dojke (99). Cimetidin također povisuje koncentraciju PRL, no neka objavljena istraživanja nisu ukazala na povezanost s rakom dojke (100). Kod žena s prolaktinomom je koncentracija PRL bila jako povišena. Međutim, samo je nekoliko prijavljenih slučajeva raka dojke kod žena ili muškaraca s prolaktinomom (101,102). Do sada je provedeno samo jedno manje kohortno istraživanje sa 67 ispitanica s prolaktinomom (103). Podaci jednog nedavno objavljenog istraživanja ukazali su na umjerenu pozitivnu povezanost između prolaktina i rizika od raka dojke među ženama od kojih je većina bila u menopauzi; međutim, potrebni su daljnji kontrolni pregledi kako bi se pojačala statistička snaga testa za analize u podskupinama (104).

Nedavno su u jednom velikom višeetničkom kohortnom ispitivanju procijenjene genetičke varijacije u genima PRL i receptora PRL (PRLR) kao predskazateljima koncentracije PRL u plazmi i rizika od raka dojke među populacijom američkih crnkinja, rođenih Havajćanki, američkih Japanki, latinoamerikanki i bjelkinja (105). U toj opsežnoj analizi, koja je pokrivala 59 kb lokusa PRL i 210 kb lokusa PRLR, nije bilo statistički značajne povezanosti između uobičajene varijacije tih genskih kandidata i rizika od raka dojke

Human breast cancer and PRL – *in vivo* investigations

Epidemiology of PRL and human breast cancer

Several epidemiological studies have indicated that PRL may also function as a progression factor for human breast cancer (8,91-93). A number of studies have evaluated the association between PRL levels and several well-confirmed breast cancer risk factors such as parity and age at first birth, age at menarche and menopause, family history of breast cancer, mammographic density, ethnic differences, dietary intake, medication use, and prolactinomas.

PRL levels appear to decrease, at least modestly, with each additional pregnancy (94,95). Also, no independent association between age at first birth and PRL level was observed (94). Overall, no significant associations between PRL and either age at menarche or age at menopause were reported (94,96). In a subset of subjects at risk with family history of breast cancer, basal serum PRL levels were significantly elevated (11). However, several other studies (94-96) offered scanty data to support association of the family history of breast cancer with PRL concentration.

Increased PRL levels were observed in postmenopausal women with increased breast tissue density (94,97), suggesting a measurable influence of PRL on breast epithelial and/or stromal proliferation.

Several studies evaluated PRL levels and dietary intake (95,98) but consistent findings are yet to be reported.

A number of medications are known to increase (e.g., oral contraceptives, reserpine, haldol, cimetidine, and the phenothiazines) or decrease (e.g., levodopa) plasma PRL levels. Long-term use of oral contraceptives increases the risk of breast cancer (99). Cimetidine also increases PRL levels, but a few studies published have not shown any meaningful link with breast cancer (100). Women with prolactinomas have greatly elevated PRL levels. However, only a few case reports of breast cancer in women or men with prolactinomas (101,102) and a small cohort study of 67 women with prolactinomas (103) have been published to date. Data of one recent study suggest modest positive association between prolactin and breast cancer risk among predominately premenopausal women; however, further follow-up is needed to increase statistical test power for subgroup analyses (104).

Genetic variations in PRL and PRL receptor (PRLR) genes as predictors of plasma PRL levels and breast cancer risk among African-American, native Hawaiian, Japanese-American, Latin, and white women were evaluated recently in a large multiethnic cohort study (105). In this comprehensive analysis covering 59 kb of the PRL locus and 210 kb of the PRLR locus, no significant association was found between common variation in these candida-

ili koncentracije PRL u plazmi. Neravnoteža povezanosti (engl. *linkage disequilibrium*, LD) između PRL i PRLR u toj višeetničkoj populaciji ponudila je okvir za istraživanje tih gena u odnosu na ishode ostalih bolesti koje su povezivane s PRL kao i za opsežnija istraživanja koncentracije PRL u plazmi.

Klinički podaci o povezanosti između PRL i raka dojke

Funkcija PRL u etiologiji i napredovanju raka dojke kod ljudi nije još sasvim razjašnjena. Podaci iz objavljene literature nisu sukladni, čak su i proturječni. Međutim, postoje značajni dokazi da bi PRL mogao imati određenu ulogu u raku dojke kod ljudi.

Incidencija hiperprolaktinemije bila je značajno viša kod bolesnika s metastatskim rakom dojke nego kod bolesnika s nemetastatskim rakom ili s mastopatijom ili s uznapredovalim solidnim tumorom različite histologije (106). Hiperprolaktinemija je gotovo jedino bila primijećena kod bolesnika s metastatskim rakom dojke tijekom napredovanja bolesti (8,107). Primijećeno je da je hiperprolaktinemija važan pokazatelj nepovoljne prognoze kod bolesnika oboljelih od raka dojke sa zahvaćenim čvorovima (108). Rezultati jednoga drugog istraživanja ukazuju na moguću povezanost hiperprolaktinemije i preveliке ekspresije p53 s agresivnošću tumora, ranijim pogoršanjem zdravstvenog stanja ili pojavom metastaza ili lošom ukupnom stopom preživljavanja kod bolesnika oboljelih od raka dojke bez zahvaćenih čvorova (9). Kod bolesnika s rakom dojke, odnosno s primarnim tumorima stupnja II i III se nakon povećanja broja zahvaćenih limfnih čvorova značajno povećala učestalost PRL-pozitivnih tumora (109). S povećanjem veličine tumora primijećena je statistički značajnije češća pojava hiperprolaktinemije, kod bolesnika s hiperprolaktinemijom je rizik od razvoja povratne/metastatske bolesti bio značajno veći, a što se ozbiljnosti tiče, 8% njihovih tumora pokazalo je pozitivnu imunoreaktivnost s protutijelima PRL, što je ukazalo na činjenicu da tumori dojke proizvode PRL koji bi mogao djelovati kao lokalni promotor rasta (110).

Rezultati nekih drugih istraživanja slažu se s prethodno spomenutim rezultatima. Koncentracija cirkulirajućeg PRL mogla bi biti vrlo korisna kao dijagnostički i prognostički biljeg kod bolesnika s rakom dojke (111) te vrijedan parametar prosudbe uspješnosti liječenja bolesnika s rakom dojke (112). Hiperprolaktinemija je pokazatelj napredovanja bolesti i nepovoljne prognoze kod bolesnika s metastatskim oblikom raka dojke (113,114). Koncentracija PRL u serumu je vjerojatno izravno ovisna o veličini primarnog tumora kod bolesnika s rakom dojke, pogotovo kod onih s hiperprolaktinemijom, no to nije pojava koja je ovisna o diferencijaciji (115).

Međutim, suprotno tome, povišenje koncentracije cirkulirajućeg PRL uvjetovano operacijom bilo je povezano s duljim razdobljem preživljavanja bez bolesti kod operabilnih slučajeva raka dojke kod bolesnika s metastazama ili onih bez metastaza u pazušnoj jami (92,116).

te genes and breast cancer risk or plasma PRL levels. The LD (linkage disequilibrium) characterization of PRL and PRLR in this multiethnic population provides a framework for studying these genes in relation to other disease outcomes that have been associated with PRL, as well as for larger studies of plasma PRL levels.

Clinical data about the association of PRL with breast cancer

The function of PRL in the etiology and progression of human breast cancer is not yet clear, and literature data are not consistent but, are even contradictory. However, there is significant evidence that PRL may play a role in human breast cancer.

The incidence of hyperprolactinemia was significantly higher in patients with metastatic breast cancer than in patients with non-metastatic breast cancer, or with mastopathy, or with advanced solid tumors of different histology (106). Hyperprolactinemia was almost exclusively found in patients with metastatic breast cancer during the course of the disease (8,107). Hyperprolactinemia was found to be an important indicator of unfavorable prognosis in node-positive breast cancer patients (108). Results of another study indicated the possible association of hyperprolactinemia and overexpression of p53 with aggressiveness of the tumor, early disease relapse or metastases, and poor overall survival in node-negative breast cancer patients (9). In primary tumors of stage II and stage III breast cancer patients, there was a significant increase in the frequency of PRL-positive tumors upon increase in the number of involved lymph nodes (109). With increasing tumor size, a significantly increased incidence of hyperprolactinemia was observed, hyperprolactinemic patients had a significantly increased risk of developing recurrent/metastatic disease, and seventy-eight per cent of their tumors showed positive immunoreactivity with PRL antibody indicating that breast tumors produce PRL which may act as a major local growth promoter (110). Results of some other studies are in agreement with those mentioned above. Circulating levels of PRL might be very useful diagnostic and prognostic marker in breast cancer patients (111), and a valuable parameter to assess treatment efficacy in breast carcinoma patients (112). Hyperprolactinemia is an indicator of disease progression and poor prognosis in metastatic breast cancer patients (113,114). Serum levels of PRL probably directly depend on the size of primary tumor in breast cancer patients, especially in those with hyperprolactinemia, but this is not a differentiation-dependent phenomenon (115).

In contrast, a surgery-induced rise in circulating PRL was associated with prolonged disease-free survival in operable breast carcinoma patients with or without axillary metastases (92,116).

Konstitutivni onkogenski nizvodni prijenos signala ErbB2 i Ras stabilizira PRLR preko inhibicijske fosforilacije glikogenske sintaze-kinaze-3-beta (GSK3 beta) na Ser9. Važno je da inaktivacija GSK3-beta korelira s povišenom koncentracijom proteina PRLR u kliničkim uzorcima tkiva raka dojke kod ljudi (39).

Povišena koncentracija prolaktina često se povezuje sa smanjenom koncentracijom spolnih hormona. Povezanost hiperprolaktinemije s reproduktivnim poremećajima, amenorejom i neredovitim menstrualnim ciklusima već je poznata od prije. Hiperprolaktinemija kod žena prije menopauze uzrokuje hipogonadizam koji se manifestira neplodnošću, oligomenorejom ili amenorejom i rjeđe galaktorejom (117). Kod bolesnica s menstrualnim problemima koncentracija PRL je viša nego kod žena s normalnom menstrualnom funkcijom (118). Međutim, rezultati jednog nedavno provedenog istraživanja pokazuju da kod žena s hiperprolaktinemijom i seksualnom disfunkcijom nije bilo hormonskih promjena u koncentraciji spolnih hormona (119). Nadalje, postoji mnogo proturječnosti s obzirom na povezanost ženskih spolnih steroida i raka. Ponovna procjena rezultata ranijih istraživanja koja podržavaju kancerogeni kapacitet estrogena otkrila je mnogo nedostataka i proturječnosti. Nedavno su klinička istraživanja hormonske nadomjesne terapije kod žena u postmenopauzi opravdale povoljan antikancerogeni učinak na nekoliko organa, uključujući i dojke kod žena. Novootkrivena povezanost nedostatka estrogena i rizika od raka usne šupljine također proturječi tradicionalnom konceptu raka uzrokovanog estrogenom. Međutim, karcinomi organa koji u visokoj mjeri ovise o estrogenu, kao što su dojke, endometrij i jajnici pojavljuju se u razdoblju prije i poslije menopauze. Usprkos različitim epidemiološkim podacima o tim dvjema skupinama raka, mehanizam poremećaja u regulaciji gena u podlozi inicijacije tumora ne može djelovati kroz potpuno oprečne putove (120). To ukazuje da je organima koji su u blažoj mjeri ovisni o estrogenu njegov ozbiljan nedostatak dovoljan za poremećaj u regulaciji gena, dok je organima kod kojih je ta ovisnost velika za to dovoljan čak i blagi nedostatak estrogena. Ti novi rezultati o raku povezanom s pušenjem te s hormonima mogu dovesti do istog obrata; ne estrogen, nego pomanjkanje estrogena može izazvati inicijaciju raka (120).

Inhibicija aktivnosti PRL

Smanjenje koncentracije PRLR farmakološkim ili genetičkim sredstvima u stanicama raka dojke kod ljudi dramatično smanjuje transformaciju i kancerogena svojstva tih stanica (121). Tamoksifen (TAM), terapija prvog izbora kod bolesnika s rakom dojke pozitivnim na receptore estrogena (ER-pozitivne) u razdoblju prije ili poslije menopauze, ima također antiprolaktinsko djelovanje (122). Antilaktogenska aktivnost TAM rezultat je njegove interakcije s antilaktogenskim veznim mjestom (engl. *antilactogen binding site*, ALBS) (123) koje je smješteno na recep-

Constitutive oncogenic signaling downstream of ErbB2 and Ras stabilizes PRLR via inhibitory phosphorylation of glycogen synthase kinase 3 beta (GSK3 beta) on Ser9. Importantly, inactivation of GSK3 beta correlates with elevated levels of PRLR protein in clinical human breast cancer specimens (39).

Increased prolactin levels are often associated with decreased sexual hormone levels. Association of hyperprolactinemia with reproductive disorders, amenorrhea and irregular menstrual cycles has already been known. Hyperprolactinemia in premenopausal women causes hypogonadism manifested by infertility, oligomenorrhea or amenorrhea and less often by galactorrhea (117). Patients with menstrual disturbances had higher PRL levels than women with normal menstrual function (118). However, results of one recent study have shown that no hormonal changes in serum levels of sexual hormones were found in women with hyperprolactinemia and sexual dysfunction (119). Furthermore, there are many contradictions concerning the association of female sexual steroids with cancer. Re-evaluation of earlier results supporting the carcinogenic capacity of estrogen has exhibited many shortcomings and controversies. Recently, clinical studies on hormone replacement therapy in postmenopausal women have justified beneficial anticancer effects in several organs, even in the female breast. The newly revealed association between estrogen deficiency and oral cancer risk also means a contradiction with regard to the traditional concept of estrogen-induced cancer. However cancers of highly estrogen dependent organs such as breast, endometrium and ovary exhibit both premenopausal and postmenopausal occurrence. In spite of different epidemiological data of these two groups of cancers, the mechanism of gene regulation disorder in the background of tumor initiation cannot act through entirely opposite pathways (120). This suggests that serious estrogen deficiency in moderately estrogen sensitive organs, and even mild estrogen deficiency in highly estrogen dependent organs is enough to provoke gene regulation disorders. New findings both on smoking- and hormone related cancers might lead to the same reversal; estrogen deficiency rather than estrogen itself may provoke cancer initiation (120).

Inhibition of PRL action

A decrease in PRLR levels achieved by either pharmacologic or genetic means in human breast cancer cells dramatically reduced transformation and tumorigenic properties of these cells (121). Tamoxifen (TAM), the first line of therapy in pre- and postmenopausal ER (estrogen receptor)-positive breast cancer patients also has an anti-prolactin action (122). Antilactogenic activity of TAM results from its interaction with the antilactogen binding site (ALBS) (123) which is located on the PRL receptor.

toru PRL. ALBS pripada obitelji veznih mjesta povezanih s membranama visokog afiniteta nazvanima antiestrogenska vezna mjesta (engl. *antiestrogen binding sites*, AEBS) (124). Djelujući preko ALBS, antiestrogeni sprječavaju rast stanica koje odgovaraju na PRL čak i u odsutnosti ER (125,126). Rezultati ovih istraživanja upućuju da je ALBS u receptoru PRL i da TAM i drugi srodni antiestrogeni mogu spriječiti rast ER-negativnih stanica ljudskog raka dojke *in* vitro pomoću tog mehanizma (34). Ti podaci također ukazuju da bi se TAM i drugi srodni lijekovi koji djeluju na razini ciljanog tkiva mogli klinički koristiti u liječenju bolesnika s hiperprolaktinemijskim rakom dojke. Međutim, raloksifen, selektivni modulator estrogenskog receptora i time i agens za sprječavanje raka dojke, nema značajnijeg učinka na koncentraciju PRL kod žena u razdoblju prije menopauze s visokim rizikom za raka dojke (127).

Zaključak

PRL sintetiziraju ljudske stanice raka dojke u kulturi, a djeluje u autokrinoj/parakrinoj petlji unutar tkiva dojke. I zdrave i maligne stanice dojke sadrže duge i kratke oblike PRLR. PRL stimulira citoskeletnu reorganizaciju i pokretnost stanica raka dojke. PRL može također utjecati na stvaranje raka dojke na način da modulira vaskularizaciju. O ulozi prolaktina kod raka dojke u glodavaca postoje brojni objavljeni dokazi opširna objavljena dokumentacija. Nekoliko epidemioloških istraživanja ukazalo je da bi PRL mogao funkcionirati kao čimbenik napredovanja raka dojke kod ljudi. Hiperprolaktinemija je pronađena gotovo isključivo kod bolesnika s metastatskim rakom dojke tijekom napredovanja bolesti, te je ustanovljeno da je važan pokazatelj nepovoljne prognoze kod bolesnika s rakom dojke i zahvaćenim limfnim čvorovima. Uzimajući sve ove podatke u obzir, može se zaključiti da je i endokrini i autokrini PRL uključen u nastanak raka dojke kod ljudi. Međutim, još je potrebno mnogo rada kako bi se razumjeli signalni putovi koje koristi PRL kako bi pospješio stvaranje tumora u stanicama dojke i interakcije tih signalnih kaskada i njihovih kompleksnih regulacijskih petlji s različitim onkogenima, čimbenicima rasta i hormonima važnima za razvoj raka u tkivu dojke. Nadalje, aktualan razvoj antagonista specifičnih za PRLR može iznjedriti nove terapijske strategije u liječenju raka dojke kod ljudi koje se temelje na blokiranju aktivnosti PRL na endokrinoj i autokrinoj/parakrinoj razini.

ALBS is a member of the family of high affinity membrane-associated binding sites called antiestrogen binding sites (AEBS) (124). Antiestrogens, acting through the ALBS, inhibit the growth of PRL-responsive cells even in the absence of ER (125, 126). The results of these studies indicate that ALBS is in the PRL receptor, and that TAM and other related anti-estrogens may inhibit the growth of ER-negative human breast cancer cells in vitro through this mechanism (34). These data also suggest that TAM and other related drugs that act at the level of target tissue may be clinically useful in the treatment of hyperprolactinemic breast cancer patients. However, raloxifene, a selective estrogen receptor modulator and thus a breast cancer prevention agent, had no significant effect on PRL levels in premenopausal women at high risk for developing breast cancer (127).

Conclusions

PRL is synthesized by human breast cancer cells in culture, and acts in an autocrine/paracrine stimulatory loop within breast tissue. Both normal and malignant mammary cells contain both long and short forms of PRLR. PRL stimulates the cytoskeletal re-organization and motility of breast cancer cells. PRL may also influence mammary carcinogenesis by modulating vascularization. Prolactin's role in rodent mammary cancer has been well documented. Several epidemiological studies have indicated that PRL may also function as a progression factor for human breast cancer. Hyperprolactinemia was almost exclusively found in patients with metastatic breast cancer during the course of the disease, and it was found to be an important indicator of unfavorable prognosis in node-positive breast cancer patients. Taking into account all these data it could be concluded that both endocrine and autocrine PRL are involved in human breast carcinogenesis. However, much more work is needed to understand the signaling pathways used by PRL to promote tumorigenesis in mammary cells, and interactions of these signaling cascades and their complex regulatory loops with different oncogenes, growth factors and hormones important in mammary carcinogenesis. Furthermore, the ongoing development of PRLR-specific antagonists may yield novel therapeutic strategies in treatment of human breast cancer based on blocking PRL actions at the endocrine and autocrine/paracrine levels.

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