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ZNAČAJ MOLEKULARNE GENETIKE ZA SKRINING KANCERA

Biljana Jekić[1]
[1] Institut za humanu genetiku, Medicinski fakultet Univerziteta u Beogradu, Beograd, Srbija

SAŽETAK

Uprkos napretku medicine, kancer je i dalje jedan od glavnih uzroka smrti u svetu. Ovo je pre svega posledica odsustva simptoma u toku progresije tumora, tako da je često u trenutku pojave simptoma bolest već u fazi kada je lečenje nemoguće. Zbog toga je razvoj specifičnih i senzitivnih metoda koje će omogućiti ranu detekciju tumora od izuzetne važnosti. Unapređenje postojećih i razvoj novih metoda molekularne genetike, uz snižavanje cene analiza, moglo bi predstavljati rešenje ovog problema. Prilikom razvoja metoda za ranu detekciju tumora treba praviti razliku da li je metoda namenjena detekciji naslednih kancerskih sindroma ili sporadičnih tumora. U slučaju naslednih kancerskih sindroma, molekularno genetičke metode se koriste da bi se detektovalo prisustvo germinativne mutacije gena za koju je poznato da je odgovorna za nastanak datog naslednog kancerskog sindroma, u cilju određivanje predispozicije za obolevanje pacijenta koji je član porodice pod rizikom. Mutacija je prisutna u svim ćelijama organizma, pa se može detektovati analizom DNK izolovane iz telesnih tečnosti ili bukalne sluzokože. Nasuprot ovome, kod sporadičnih tumora je neophodan skrining zdrave populacije u odsustvu bilo kakvih informacija o lokalizaciji tumora ili genetičkim promenama. Metodu izbora u ovom slučaju bi mogle predstavljati tečne biopsije, gde se molekularno genetičkim metodama analiziraju promene u genetičkom materijalu prisutnom u telesnim tečnostima ispitanika. Ovom metodom se tumor kod pacijenta otkriva, na primer, na osnovu promene u koncentraciji slobodne cirkulišuće DNK u krvi (cfDNA) ili na osnovu prisustva cirkulišuće tumorske DNK (ctDNA). Takođe, u skriningu za kancer se, pored genetičkih, moraju uzeti u obzir i epigenetičke promene.

Ključne reči: molekularno genetičke metode, skrining metode, nasledni kancerski sindromi, sporadični tumori

Uvod

Procenjuje se da su jedna polovina muškaraca i jedna trećina žena u riziku od dobijanja neke vrste kancera tokom života (1). Prema Svetskoj zdravstvenoj organizaciji, 30-50% svih slučajeva kancera su rezultat izloženosti poznatim faktorima rizika (UV zračenju, pušenju, zloupotrebi alkohola, gojaznosti, virusnim infekcijama itd.) i mogu se prevenirati eliminisanjem ovih faktora (2). Međutim, uprkos aktivnom pristupu koji obuhvata obrazovanje i vakcinaciju stanovništva, napredak u eliminaciji faktora rizika za nastanak kancera je spor. Čak i kada bi ovaj cilj mogao da bude postignut, veliki procenat stanovnika ostaje pod rizikom da oboli od ove bolesti. Većina smrtnih slučajeva izazvanih kancerom je posledica kasne dijagnoze bolesti (3). Rano otkrivanje bolesti, pre pojave kliničkih simptoma ili barem pre pojave metastaza, može značajno da poboljša dijagnozu i smanji troškove lečenja. Nažalost, zbog heterogenosti i složene, multifaktorske prirode kancera, metode skrininga za rano otkrivanje kancera još uvek nisu dostigle zadovoljavajuće efekte. Metode molekularne genetike, koje su moćno sredstvo za rano otkrivanje kancera, razvijaju se u dva pravca: skrining za nosioce naslednih kancerskih sindroma i skrining za pojavu sporadičnih kancera u zdravoj populaciji.

Nasledni sindromi kancera

Približno 5 do 10% svih kancera su rezultat naslednih kancerskih sindroma (4). Pacijenti sa ovim sindromima su nosioci mutacija u tumor supresorskim genima, genima koji su odgovorni

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THE IMPORTANCE OF MOLECULAR GENETICS FOR CANCER SCREENING

Biljana Jekic[1]
[1] Institute of Human Genetics, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

SUMMARY

Despite the advance of medicine, cancer remains among the leading causes of deaths worldwide. The main reason is the progression of the disease without the symptoms until the untreatable stages are achieved. To prevent this, the development of new, more sensitive and specific screening methods for early detection of cancer is necessary. Molecular genetics, through the improvement of existing and development of new methods and analysis cost reduction, could provide tools for the achievement of this goal. There are essential differences in approach regarding the purpose of screening methods: screening for hereditary cancer syndromes or sporadic cancers. In the case of hereditary cancer syndromes, molecular genetics methods are used to search for germline mutations in defined genes, to establish a final diagnosis or to estimate the risk of cancer for the patient that is identified as a member of a family in risk. The mutation is present in all cells of the organism and can be detected through non-invasive analysis of DNA from body fluids or the buccal swab. Development of molecular genetics method for screening for sporadic cancers in a healthy population, without any knowledge of cancer location or genetic change, could rely on the search of tumor genetic material in body liquids (liquid biopsies). In this case, the search for change in circulating cell-free DNA (cfDNA) concentration or genetic and epigenetic changes in circulating tumor DNA (ctDNA) in a blood sample could reveal development of a tumour. Additionally, epigenetic changes should also be considered in screening for cancers.

Keywords: molecular genetics method, screening methods, hereditary cancer syndromes, sporadic cancers

Introduction

It has been estimated that one-half of men and one-third of women are at risk throughout life from developing some type of cancer (1). According to the World Health Organization, 30-50% of all cancer cases are the result of exposition to known risk factors (UV radiation, tobacco smoking, alcohol misuse, obesity, viral infections etc.) and can be prevented through the exclusion of these factors (2). Nevertheless, despite active approach that includes education and vaccination of populations, the progress of elimination of cancer risk factors from the human populations is slow. Even if this goal could be achieved, the huge percent of the population remains at risk of developing the disease. The majority of deaths due to cancer are the result of late diagnosis of the disease (3). Early detection of disease, before clinical appearance of symptoms or at least before metastasis, can significantly improve diagnosis and reduce treatment costs. Unfortunately, because of heterogeneity and complex, multi-factorial nature of cancer, screening for cancer has not reached satisfying effects yet. Molecular genetics methods, as powerful tools for early cancer detection, are developing in two directions: screening for carriers of hereditary cancer syndromes and screening for occurrence of sporadic cancers in healthy population.

Hereditary cancer syndromes

Approximately 5% to 10% of all cancers are the result of hereditary cancer syndromes (4). Patients with these syndromes are carriers of mutations in tumor suppressor genes, genes

za ispravljanje oštećenja molekula DNK, ili ređe u protoonkogenima. Nasledni kancerski sindromi imaju mendelijanski tip nasleđivanja, sa 50% verovatnoće prenosa mutiranog gena na potomstvo pacijenta koji nosi mutaciju. Takođe, u najvećem broju slučajeva se mutacije ispoljavaju po dominantnom tipu sa visokom penetrantnošću mutiranog gena, iako postoje primeri recesivnog nasleđivanja, kao što je MAP (engl. *MUTYH-associated polyposis*) što je rezultat recesivne mutacije u *MUTYH* genu. Poznavanje načina nasleđivanja i molekularnih promena koje su u osnovi određenog kancerskog sindroma omogućava identifikaciju članova porodice koji su nosioci mutacije povezane sa kancerom. Iako su ovi pacijenti pod visokim rizikom od razvijanja kancera u ranoj dobi, njihov nadzor nakon dijagnoze značajno poboljšava njihovu prognozu.

Pored toga, s obzirom da je mutacija koja izaziva bolest prisutna u svim ćelijama organizma, može se otkriti uz pomoć neinvazivne analize DNK koja se izoluje iz telesnih tečnosti ili bukalne sluzokože. Tako, na primer, iako je svega 5% svih slučajeva raka dojke rezultat mutacije *BRCA1* i *BRCA2* gena (nasledni rak dojke), većina naslednih kancera dojke je rezultat mutacija ova dva gena (5). *BRCA1* i *BRCA2* geni se klasifikuju kao tumor supresorski geni, sa proteinskim produktima koji su uključeni u odgovor na oštećenje DNK (6). Šansa da ženski nosilac mutacije dobije rak dojke je otprilike pet puta veća u odnosu na osobu koja ne nosi datu mutaciju (5). Verovatnoća da ženski nosioci dobiju rak jajnika je povećana do 40% (7,8) u poređenju sa osobama koje nisu nosioci. Zbog germinativne prirode mutacije, ove osobe su u riziku, iako manjem, od pojave kancera na drugim organima (rak pankreasa, želuca, debelog creva, bubrega, melanoma) (9). Pored toga, muški nosioci mutacije *BRCA1* gena imaju povećan rizik da obole od raka prostate (9), a nosioci *BRCA2* mutacije od raka dojke (4). Na osnovu dobro postavljenih kriterijuma, članovi porodice sa rizikom mogu lako biti prepoznati, potvrđeni uz pomoć analize *BRCA1/2* mutacija i uključeni u definisane protokole za praćenje.

U slučaju Linčovog sindroma (nasledni nepolipozni kolorektalni kancer – HNPCC), najčešće su uzrok nastanka kancera germinativne mutacije gena koji su uključeni u popravke oštećenja na molekulu DNK (obično *MLH1*, *MSH2*, *MSH6* i *PMS2*). Mutacije ovih gena su česte i prisutne su kod jedne od 5000 osoba (10). Takođe, penetrantnost mutiranih gena je izuzetno visoka, sa verovatnoćom od 80% da se kod nosioca razvije bolest (11). Jedna od posledica mutacija *MMR* gena je genetička nestabilnost koja se ogleda u mikrosatelitnoj nestabilnosti kod nosioca mutacije. Pre analize sva četiri gena da bi se potvrdio Linčov sindrom, jednostavan genetski test može da otkrije prisustvo (ili odsustvo) mikrosatelitne nestabilnosti, što obezbeđujući brzo, jednostavno i jeftino uključivanje (ili isključivanje) pacijenta iz daljeg protokola.

Sporadični kanceri

Rano otkrivanje bolesti predstavlja najveći izazov u uspešnom lečenju kancera. Skrining testovi za otkrivanje različitih tipova tumora su od ključne važnosti za postizanje ovog cilja. Nažalost, za mnoge vrste kancera, razvoj takvih testova je zahtevan zbog inter i intra-tumorske heterogenosti, asimptomatske prirode bolesti u ranim fazama, potrebe za čestim testiranjem cele populacije i mnogih drugih faktora (epidemioloških itd.) (12,13). Da bi bio široko prihvaćen, od novog skrining testa se očekuje da ima veću specifičnost i senzitivnost i da bude manje invazivan i skup u odnosu na dostupne testove.

Razvojem tehnika molekularne genetike i sticanjem znanja o različitim tumorima (npr. *Cancer Genome Atlas project, International Cancer Genome Consortium*), tečne biopsije bi mogle da postanu testovi izbora. Tečna biopsija je test koji se radi na uzorku krvi da bi se otkrile specifične promene u slobodnoj cirkulišućoj DNK, ćelije kancera koje cirkulišu u krvi ili delovi DNK iz tumorskih ćelija u krvi (14). Delovi DNK koji cirkulišu u krvi zovu se slobodno cirkulišuća DNK (cfDNA). cfDNA potiče od ćelija koje prolaze kroz apoptozu ili nekrozu (15,16) i nalazi se u obliku DNK fragmenata veličine od 180 do 200bp (17). Iako postoje dokazi da je nivo cfDNA povećan kod nekih kancera (18-20), zbog širokog opsega cfDNA koncentracije kod pacijenata, koji može da varira od nekoliko jedinica do više hiljada jedinica ng/ml, često se detektuje preklapanje koncentracija cfDNA kod pacijenata sa tumorom i zdrave populacije

responsible for DNA repair or, less often, in proto-oncogenes. Cancer syndromes have the Mendelian type of inheritance, with a 50% transmission probability of a mutated gene on the offspring of a patient carrying mutation. The pattern of inheritance is usually dominant with high penetrance of the mutated gene, although there are also examples of recessive inheritance, like MAP (MUTYH-associated polyposis) that is the result of a recessive mutation in MUTYH gene. Knowledge about the pattern of inheritance and molecular changes underlying specific cancer syndrome provides tools for the identification of family members that are carriers of a cancer-related mutation. Although these patients are at a high risk of developing cancer from an early age, their surveillance after diagnosis significantly improves their prognosis.

Additionally, because the disease-causing mutation is present in all cells of the organism it can be detected through non-invasive analysis of DNA isolated from body fluids or the buccal swab. For example, 5% of all breast cancer cases are the result of a mutation in BRCA1 or BRCA2 gene (hereditary breast cancer) and additionally, the majority of hereditary breast cancers are the result of mutations in these two genes (5). These genes are classified as tumour suppressor genes, with protein products involved in response to DNA damage (6). A chance for developing breast cancer for a female carrier of mutation is approximately five times higher compared with non-carrier (5). Female carriers have up to 40% raised probability to develop ovarian cancer, compared to non-carriers (7,8), and, because of germline nature of the mutation, less often in some other organs (pancreatic cancer, stomach cancer, colon, kidneys, melanoma) (9). Additionally, males BRCA1 mutation carriers are in risk of developing prostate cancer (9), and BRCA2 mutation carriers of breast cancer (4). According to well-established inclusion criteria, family members at risk can easily be recognized, confirmed through analysis of BRCA1/2 mutations and included in defined follow-up protocols.

In the case of Lynch syndrome (hereditary nonpolyposis colorectal cancer – HNPCC), germline mutations in mismatch repair genes (usually MLH1, MSH2, MSH6 and PMS2) result in the development of the disease. Mutations of these genes are common, with the incidence of one in 5000 people (10), and penetrance of the mutated gene is extremely high, with a probability of 80% for the carrier to develop the disease, compared to non-carriers (11). One of the consequences of mutations in MMR genes is genetic instability that is reflected in the form of microsatellite instability in the carrier of the mutation. Before sequencing of all four genes to confirm Lynch syndrome, a simple genetic test can reveal the presence (or absence) of microsatellite instability thus enabling quick, simple and cheap inclusion (or exclusion) of the patient in the further protocol.

Sporadic cancers

The main challenge in the successful treatment of cancer is the early detection of disease. For the achievement of this goal, screening tests for the detection of different types of tumours are pivotal. Unfortunately, for many types of cancer the development of such a test is demanding, because of inter and intra-tumour heterogeneity, asymptomatic nature of the early disease, the necessity for frequent assessment of the screening group, and many others factors (epidemiological etc.) (12,13). To be widely accepted, the new screening test is expected to have higher specificity and sensitivity and to be less invasive and expensive in comparison with available tests.

With the development of molecular genetics techniques and accumulation of knowledge about different tumours (e.g. Cancer Genome Atlas project, International Cancer Genome Consortium) liquid biopsies could become the tests of choice. Liquid biopsy is a test done on a sample of blood to look for cancer cells from a tumour that are circulating in the blood or for pieces of DNA from tumour cells that are in the blood (14). Pieces of DNA circulating in the blood are termed circulating cell-free DNA (cfDNA). cfDNA originates from cells that are undergoing apoptosis or necrosis (15,16) and is presented in the form of 180-200bp long DNA fragments (17). Although there is evidence that level of cfDNA is elevated in some cancers (18-20), overlapping between the concentration of cfDNA in patients with tumour and healthy population has been often reported, due to wide range of cfDNA concentration in

(21,22). Stoga su potrebna dalja istraživanja kako bi se omogućila primena ovih nalaza u skrining testovima.

Kod pacijenata sa kancerom deo cfDNA potiče iz tumorskih ćelija i ova frakcija se zove slobodno cirkulišuća tumorska DNK (ctDNA). Nasuprot cfDNA, ctDNA može biti oslobođena iz živih tumorskih ćelija (23). Jedna od najvećih prednosti analize ctDNA je da se uz pomoć ove tehnike postiže slučajno uzorkovanje različitih ćelijskih populacija u tumoru. Analizom ctDNK mogu se detektovati genetičke i epigenetičke promene specifične za tumor i na taj način može se otkriti prisustvo bolesti mnogo pre njenog ispoljavanja. Fragmenti ctDNA su obično kraći u poređenju sa cfDNA (24). ctDNA predstavlja samo malu frakciju cfDNA (koncentracija varira od 0,1 do 10% svih cfDNA) (25), ali zbog razvoja tehnika molekularne genetike, sada je moguće analizirati i tako male količine materijala.

U skriningu zdrave populacije neophodno je tražiti dokazane, tumor-specifične promene u genomu, ili promene koje mogu biti povezane sa razvojem tumora, na primer varijacije u broju kopija (engl. *copy number variation* – CNV). Zbog potrebe da se analizira ceo genom, ove tehnike su i dalje skupe za stalno praćenje cele populacije. Zbog toga, praćenje epigenetskih promena u genomu je atraktivan pristup skrininga genoma za potencijalnu malignu transformaciju. Epigenetska promena predstavlja reverzibilnu promenu u metilaciji DNK ili hemijskoj modifikaciji proteina hromatina. Dokazano je da je hipermetilacija DNK česta u tumorima i da se javlja rano u tumorigenezi. Različiti testovi koji analiziraju epigenetske promene, kao na primer skrining za kolorektalni kancer uz pomoć detekcije metilacije gena *Septin* 9 (26) se već koriste.

Zaključak

Iako rano otkrivanje kancera značajno poboljšava dijagnozu i smanjuje troškove lečenja, heterogenost i složena, multifaktorska priroda kancera i dalje ostaju prepreka u razvoju pouzdanih i široko primenjivih metoda za skrining kancera. Očekuje se da će razvoj tehnika molekularne genetike i sticanje znanja o različitim tumorima obezbediti nove, visoko specifične i senzitivne, neinvazivne i jeftinije skrining testove u bliskoj budućnosti.

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patients that can vary from a few to thousands of ng/ml (21,22). Hence, further researches are necessary to enable the application of these findings in the screening tests.

In patients with cancer part of cfDNA originates from a tumour cell, and this fraction is termed the circulating tumour DNA (ctDNA). In contrast to cfDNA, ctDNA can be released from living tumour cells (23). One of the major advantages of ctDNA is that through this technique random sampling of different cell populations in the tumour is achieved. ctDNA fragments are usually shorter compared to cfDNA (24). The search for genetic and epigenetic changes in ctDNA that could point to the development of a tumour is a promising approach. ctDNA represents only small fraction of cfDNA (concentration varies from 0.1 to 10% of all cfDNA (25), but because of development of molecular genetics techniques, it is now possible to analyze such a small amount of material.

In the screening of healthy population, it is necessary to search for proven, tumour specific changes in genome, or for change that could be related with the development of tumour, for example copy number variation (CNV). These techniques are still expensive for continuous, whole population monitoring because of the requirement of coverage of the whole genome. Because of this, follow up of the epigenetic changes in the genome is an attractive approach of genomic screening for potential malignant transformation. Epigenetic change refers to the reversible change in DNA methylation status or chemical modifications of chromatin proteins. Hypermethylation of DNA has been proven as a common event in tumours and occurs early in tumorigenesis. Different tests that utilize this change, for example screening for colorectal carcinoma through detection of methylated Septin 9 (26), are already in use.

Conclusion

Although an early detection of cancer significantly improves diagnosis and reduces treatment costs, heterogeneity and complex, multifactorial nature of cancer still remains an obstacle in the development of reliable and widely applicable cancer screening methods. It is expected that development of molecular genetics techniques and accumulation of knowledge about different tumours will provide new, highly specific and sensitive, non-invasive and less expensive screening tests in near future.

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Autor za korespondenciju: Biljana Jekić, Institut za humanu genetiku, Medicinski fakultet Univerziteta u Beogradu, Višegradska 26, 11000 Beograd, Srbija; e-mail: jekic.b@gmail.com

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Corresponding author: Biljana Jekic, Institute of Human Genetics, Faculty of Medicine, University of Belgrade, Visegradska 26, 11000 Belgrade, Serbia; e-mail: jekic.b@gmail.com