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Naviką slopinančių genų promotoriaus DNR metilinimo tyrimai krūties navikuose

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Hypermethylation of Tumor Suppressor Genes in DNA from Breast Carcinoma

Summary

Breast cancer is the most prevalent malignancy of women in Lithuania and word-wide. The course of disease differ markedly among patients, therefore molecular characterisation of tumour is very important. However, current prognostic markers are mainly based on clinical parameters and do not enable a reliable selection of the patients with high risk of disease progression. Molecular characterisation of tumour through detection of changes in cancer-related genes can help to estimate the risk of cancer progression, detect the molecular targets for modern treatment strategies. In order to evaluate the suitability of epigenetic biomarkers for molecular characterisation of breast cancer we analysed promoter hypermethylation in a wide panel of regulatory genes involved in cell cycle control, signalling, apoptosis and DNA repair.

76 primary breast carcinomas of early stage (pT1-2) were selected for the study. Aberrant methylation in promoter regions of seven tumour suppressor genes (*p16, p14, RARβ, RASSF1A, DAPK, GSTP1* and *MGMT*) was analysed by means of methylation-specific PCR. The "Real Time" PCR method was adapted for detection of epigenetic changes in circulating tumour DNA from plasma of cancer patients.

Most of the early-stage breast tumours (63/76) exhibited hypermethylation in at least one gene involved in analysis. The overall sensitivity of the epigenetic biomarkers was 83%. Gene *RASSF1A* was the most frequently (56 of 76 cases) hypermethylated gene in breast tumours. Statistical analysis revealed significant associations between expression of hormonal receptors (ER and PR) and hypermethylation of gene *RASSF1A*, expression of HER-2 and hypermethylation of gene *GSTP1*, hypermethylation of gene *RARβ* and poor differentiation (G2-3) of the tumours. Our study shows a significant involvement of epigenetic changes in the early stage breast carcinomas. After thorough analysis in the bigger groups of patients a set of analysed biomarkers can be used as the molecular biomarkers of breast cancer.

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Santrauka

Krūties vėžys yra dažniausia Lietuvos ir viso pasaulio moterų onkologinė liga. Ši liga pasižyminti nevienoda eiga, todėl molekulinė vėžio analizė yra labai svarbi. Šiuo metu ligos eiga prognozuojama, remiantis riboto informatyvumo klinikinių žymenų sistema, o gydymui tik pavieniais atvejais skiriami atrankūs vaistai, nukreipti į ligą sukėlusį genetinį pakitimą. Naviko molekulinė analizė padeda aptikti pažaidas vėžio genuose ar jų raiškos pakitimus ir informuoja apie ligos išsivystymo priežastis, padeda prognozuoti ligos progresavimo tikimybę, atskleidžia taikinius naujos kartos gydymo priemonėms. Siekiant įvertinti krūties vėžio epigenetinių biožymenų efektyvumą mes tyrėme reguliacinių genų, dalyvaujančių ląstelės ciklo kontrolėje, signalų perdavime, apoptozėje ir DNR reparacijoje, promotoriaus sekų hipermetilinimą.

Tyrimui buvo atrinktos 76 pirminės ankstyvos stadijos (pT1-2) krūties karcinomos. Metilinimo pakitimai promotoriaus sekoje buvo tiriami septyniuose naviką slopinančiuose genuose (*p14, p16, RARβ, RASSF1A*, *DAPK*, *GSTP1* ir *MGMT*), taikant metilinimui jautrią PGR. „Tikro laiko“ PGR metodas buvo įdiegtas epigenetinių pakitimų tyrimams cirkuliuojančioje vėžio DNR, išskirtoje iš ligonių kraujo plazmos.

Didžioji dalis ankstyvos stadijos krūties karcinomų (63/76) turėjo bent vieno tirto geno hipermetilinimą. Bendras epigenetinių žymenų informatyvumas – 83%. Dažniausiai hipermetilinimas krūties karcinomose nustatytas gene *RASSF1A* (56/76). Statistinė analizė parodė patikimą arba artimą patikimai asociaciją tarp hormonų receptorių raškos ir geno *RASSF1A* hipermetilinimo, HER-2 raiškos ir geno *GSTP1* hipermetilinimo, bei geno *RARβ* hipermetilinimo ir blogos diferenciacijos (G2-3) navikų. Atliktas tyrimas parodė, kad ankstyvos stadijos krūties navikų patogenezė glaudžiai susijusi su epigenetinėmis genų pažaidomis. Dalis tirtų epigenetinių žymenų po detalesnės analizės galėtų būti įtraukti į krūties vėžio molekulinių žymenų sistemą.

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