Gene 580 (2016) 159–168

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Artificial neural network-based exploration of gene-nutrient interactions in folate and xenobiotic metabolic pathways that modulate susceptibility to breast cancer☆

Shaik Mohammad Naushad [a,*], M. Janaki Ramaiah [a], Manickam Pavithrakumari [a], Jaganathan Jayapriya [a], Tajamul Hussain [b], Salman A. Alrokayan [c], Suryanarayana Raju Gottumukkala [d], Raghunadharao Digumarti [e], Vijay Kumar Kutala [f]

[a] School of Chemical & Biotechnology, SASTRA University, Tirumalaisamudram, Thanjavur 613401, India
[b] Center of Excellence in Biotechnology Research, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia
[c] Department of Biochemistry, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia
[d] Department of Surgical Oncology, Nizam's Institute of Medical Sciences, Hyderabad -500082, India
[e] Department of Medical Oncology, Nizam's Institute of Medical Sciences, Hyderabad 500082, India
[f] Department of Clinical Pharmacology and Therapeutics, Nizam's Institute of Medical Sciences, Hyderabad 500082, India

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 1 January 2016
Accepted 12 January 2016
Available online 16 January 2016

Keywords:
Breast cancer
Folate pathway
Xenobiotic pathway
Artificial neural network
Methylome

ABSTRACT

In the current study, an artificial neural network (ANN)-based breast cancer prediction model was developed from the data of folate and xenobiotic pathway genetic polymorphisms along with the nutritional and demographic variables to investigate how micronutrients modulate susceptibility to breast cancer. The developed ANN model explained 94.2% variability in breast cancer prediction. Fixed effect models of folate (400 μg/day) and B_{12} (6 μg/day) showed 33.3% and 11.3% risk reduction, respectively. Multifactor dimensionality reduction analysis showed the following interactions in responders to folate: RFC1 G80A × MTHFR C677T (primary), COMT H108L × CYP1A1 m2 (secondary), MTR A2756G (tertiary). The interactions among responders to B_{12} were RFC1G80A × cSHMT C1420T and CYP1A1 m2 × CYP1A1 m4. ANN simulations revealed that increased folate might restore ER and PR expression and reduce the promoter CpG island methylation of extra cellular superoxide dismutase and BRCA1. Dietary intake of folate appears to confer protection against breast cancer through its modulating effects on ER and PR expression and methylation of EC-SOD and BRCA1.

The etiology of breast cancer is complex involving several physiological, genetic, environmental, and epigenetic factors (Petrakis, 1977; McPherson et al., 2000). Given the high incidence of breast cancer in women, specific emphasis was given on the estrogen metabolism as catechol estrogens were reported to form adducts with DNA, which may contribute to induce mutagenicity (Cavalieri and Rogan, 2011). On the other hand, methoxy estrogens were found to be protective (Dawling et al., 2003). The phase I enzymes belong to cytochrome P450 super family and convert estrogens to catechol estrogens (Hachey et al., 2003). Certain genetic variants of CYP1A1, i.e. CYP1A1 m1, CYP1A1 m4 were reported to induce high expression of CYP1A1 leading to increased catechol estrogen production (Naushad et al., 2011a). The phase II enzymes detoxify catechol estrogens by the following mechanisms: i) O-methylation of catechol estrogens catalyzed by catechol-o-amine methyl transferase (COMT); ii) conjugation of semiquinones/quinones (formed by catechol estrogens) with glutathione in the presence of GSTs (Chen et al., 2004). The COMT H108L and GSTT1/GSTM1 null variants hamper this detoxification process thus exerting the breast cancer risk (Naushad et al., 2011b).

The methylation of catechol estrogens further depends on the bioavailability of S-adenosylmethionine (SAM) (JE1 et al., 2002), a byproduct of one-carbon metabolism (Inoue-Choi et al., 2012). The dietary folate in the form of folylpolyglutamate enters the intestine and undergoes hydrolysis to form folylmonoglutamate by the action of glutamate carboxypeptidase II (GCPII) and thus gets absorbed by the intestine. Folate reductase catalyzes the two-step reduction of folate to form dihydrofolate (DHF) and tetrahydrofolate (THF). The THF from the plasma is transported to RBC with the help of reduced folate

Abbreviations: GCPII, Glutamate carboxypeptidase II; RFC1, Reduced folate carrie 1; cSHMT, Cytosolic serine hydroxymethyl transferase; TYMS, Thymidylate synthase; MTHFR, 5,10-methylene tetrahydrofolate reductase; MTR, Methionine synthase; MTRR, Methionine synthase reductase; SAM, S-adenosyl methionine; SAH, S-adenosyl homocysteine; CYP, Cytochrome P450; COMT, Catechol-o-amine methyl transferase; GST, Glutathione-S-transferase; RASSF1, Ras association (RalGDS/AF-6) domain family member 1; BRCA1, Breast cancer 1,early onset; RARB1, Retinoic acid receptor, beta; EC-SOD, Extracellular superoxide dismutase; ANN, Artificial neural network; MDR, Multifactor dimensionality reduction analysis.

☆ Conflicts of interest statement: All the authors hereby declare no conflicts of interest.

* Corresponding author at: School of Chemical and Biotechnology, SASTRA University, Tirumalaisamudram, Thanjavur 613401, India.
E-mail address: naushadsm@gmail.com (S.M. Naushad).

http://dx.doi.org/10.1016/j.gene.2016.01.023
0378-1119/

carrier 1 (RFC1). THF accepts methylene moiety from serine and forms 5,10-methylene THF in the presence of cytosolic serine hydroxymethyltransferase (cSHMT). 5,10-methylene THF is the substrate for two enzymes, i.e. thymidylate synthase (TYMS) and methylene tetrahydrofolatereductase (MTHFR), which catalyze the conversion of dUMP to dTMP, and FAD-dependent reduction of 5,10-methylene THF to 5-methyl THF. 5-methyl THF remethylates homocysteine to methionine in the presence of methionine synthase/methionine synthase reductase (MTR/MTRR) holoenzyme complex. Methionine is the precursor for the synthesis of SAM, which is a universal methyl donor that donates methyl group to DNA, catecholamines and proteins. Upon donating methyl group, SAM is converted to S-adenosyl homocysteine (SAH), which gives back homocysteine through hydrolysis (Naushad et al., 2011a).

Thiamine and cofactors of folate pathway, namely folate, riboflavin, and vitamin B6, were reported to confer protection against breast cancer (Cancarini et al., 2015). Several functional polymorphisms in folate pathway have been investigated by various researchers for their possible association with breast cancer (Stevens et al., 2007). In our earlier study, we reported positive association of RFC1 G80A and MTRR A66G and inverse association of cSHMT C1420T with breast cancer (Mohammad et al., 2011). We have also showed the cross-talk between the folate and xenobiotic metabolic pathways modulating the breast cancer risk (Naushad et al., 2011b). The folate pathway also plays a pivotal role in DNA methylation and thus any deregulation might induce hypermethylation of tumor suppressors and hypomethylation of proto-oncogenes, which are hallmarks of cancer (Naushad et al., 2012a).

Dietary folate and cobalamin intake were shown to exhibit inverse association with methylation of breast cancer 1,early onset (BRCA1), and retinoic acid receptor, beta (RARB1) (Pirouzpanah et al., 2015). Breast cancer patients with plasma folate levels in the highest tertile were reported to have less risk for mortality compared to those with plasma folate in the lowest tertile (McEligot et al., 2015). Mediterranean diet rich in cofactors of folate pathway was shown to reduce breast cancer risk in subjects with MTHFR 677 T and MTR 2756 A variant alleles (Kakkoura et al., 2015). Higher dietary folate intake was shown to reduce risk for the ER-negative breast cancer in pre-menopausal women (de Batlle et al., 2014). BRCA1 methylation in all types of breast cancers and Ras association (RalGDS/AF-6) domain family member 1 (RASSF1) methylation in the ER/PR-negative breast cancers was reported to correlate positively with total plasma homocysteine (Naushad et al., 2014).

In the current study, we have aimed to develop a risk prediction model for breast cancer by incorporating demographic data, family history, dietary intake of folate, B_2, B_6, B_{12} along with data on the fourteen polymorphisms of folate and xenobiotic metabolic pathways. The rationale of this risk prediction model was to assess the influence of life style modulation in bringing down breast cancer risk, specifically by modulating micronutrient intake. In parallel, we have studied folate-mediated changes in methylome at RASSF1, BRCA1, (BCL2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3), extracellular superoxide dismutase (EC-SOD) loci.

1. Materials and methods

1.1. Sample size calculation

Based on our previous study, the difference in means of plasma folate levels between controls vs. basal-like breast cancer was 0.62, standard deviation was 1.2, the ratio of controls to basal-like breast cancer was 5.0. Hence, 36 basal-like breast cancers and 240 healthy controls were required to obtain 80% power with type I α error of 0.05. The incidence of basal-like breast cancer in India was reported to vary from 12.5% to 29.8%. Hence, assuming the incidence to be around 12.5%, the required number of total breast cancer samples was calculated as 288.

1.2. Recruitment of subjects

We have conducted a case–control study by recruiting 342 breast cancer patients and 253 normal healthy controls in the Departments of Medical and Surgical Oncology, Nizam's Institute of Medical Sciences, Hyderabad, India, during the period of June 2009 to June 2012. The diagnosis of breast cancer was based on the mammogram and histopathological examination of the biopsy. The inclusion criteria were i) patients aged between 18 and 70 yr. with confirmed diagnosis of breast cancer based on mammography and histopathological examination; ii) controls matched with cases in terms age, ethnicity, and geographical location with no history of any benign or malignant breast disease; and iii) subjects willing to give informed consent. The exclusion criteria were i) patients with any co-morbid disorder or malignancy; ii) cases already under radiation and chemotherapy will be excluded from methylation studies; and iii) patients whose medical records are not accessible. The Institutional Ethical Committee of Nizam's Institute of Medical science (NIMS), Hyderabad India, has approved the study protocol (EC/NIMS/767/2007, dated 05.09.2008). The informed consent was obtained from all the subjects.

1.3. Measurements

From all the subjects, the demographic characteristics such as age (yr), body mass index (BMI, kg/m^2), age of menarche (yr), parity, and the menopausal status (pre-/post-menopausal) were recorded during personal interviews conducted by a team of trained researchers. To calculate body mass index, height and weight were recorded to the nearest measurement of 0.1 cm and 0.1 kg, respectively. The estrogen exposure time was calculated based on the age of menarche and age of menopause (post-menopausal)/age at the time of sample collection (pre-menopausal).

1.4. Dietary assessment

All the participants were asked to complete a dietary record of all the food items consumed and their quantity for 4-day period from Thursday to Sunday as this method is validated and being followed by National Diet and Nutrition Survey in the United Kingdom. The data obtained were segregated into white vegetables, green vegetables, leafy vegetables, fruits, milk products, and non-vegetarian foods. The dietary intake of folate, vitamins B_2, B_6, and B_{12} was assessed based on these diaries. The nutritive value of Indian foods (Gopalan et al., 1989), McCance and Widdowson's the composition of foods (Krebs, 2002), and the United States Department of Agriculture's National Nutrient Database for Standard Reference release 18 (USDA, Washington, DC, USA) (U.S. Department of Agriculture and Agricultural Research Service., 2006) were referred to calculate micronutrient quantity per food item. Average daily nutrient intakes were calculated as grams of food multiplied by the amount of each micronutrient in the food and the frequency of consumption, summing over all the foods consumed. None of the subjects enrolled in this study were on any vitamin supplements.

1.5. Immunohistochemistry

Immunohistochemistry for ER, PR, HER2/Neu was performed on the serial sections of paraffin-embedded breast cancer tissues using the standard streptavidin–biotin complex method with 3, 3′-diaminobenzidine as the chromogen. ER antibody (Clone SP1, Lab Vision) was used at 1:250 dilution in 10 mM citrate buffer (pH 6.0) with an 8-min microwave antigen retrieval. PR antibody (Clone 1E2, Ventana) was used as per the Ventana automated stainer standard CC1 protocol. HER2 antibody (Clone SP3, Lab Vision) was used at 1:100 dilution in 0.05 M Tris buffer (pH 10.0) with heat-induced antigen retrieval at 95 °C for 30 min.

Biomarker expression from immunohistochemistry assays was scored by two pathologists, who were blinded to the clinicopathological