Introduction

OX40L (known as TNFSF4, CD252), the cognate ligand of OX40, is a member of the tumor necrosis factor superfamily. The OX40L gene is located on human chromosome 1 and encodes a type II glycoprotein which expressed not only on professional antigen-presenting cells (APCs), but also on CD4+ T cells, vascular endothelial cells, mast cells and activated NK cells [1,2,3,4,5]. The interaction between OX40 and OX40L provides a costimulatory signal that strongly regulates the proliferation and survival of T lymphocytes, modulates NKT cell and NK cell function, and contributes to the differentiation and activity of regulatory T cells [6,7]. Antitumor immunity provides a protective barrier to tumor formation and progression [8]. Accumulating evidence indicates that inflammatory response plays a decisive role at different stages of tumor development and contributes to the initiation and progression of cancer. Moreover, avoiding immune destruction was considered as an emerging hallmark of cancer [9,10].

Breast cancer is the most common invasive malignancy. It is estimated that more than one million women are diagnosed with breast cancer and over 450,000 dead worldwide every year [11]. In the past decades, several studies have suggested that OX40L involved in the initiation and progression of breast cancer. OX40L fusion protein significantly inhibited the growth mouse 4T1 breast tumor model [12] and breast cancer cell-derived thymic fusion protein significantly inhibited the growth of mouse 4T1 breast tumor model [13]. Interestingly, some results demonstrated gender-specific effects in the associations of OX40L gene variation with the risk for breast cancer.

Single nucleotide polymorphisms (SNPs) association analysis reveals the predisposition of diverse diseases in different ethnic groups and benefits to develop effective risk assessment and treatment strategies. So far, previous studies were mainly focused on the association of OX40L polymorphisms with autoimmune diseases and inflammatory diseases such as systemic lupus erythematosus (SLE), systemic sclerosis and show gender-specific effects in some studies. Accordingly, we performed a case-control study including 557 breast cancer patients and 580 age- and sex-matched healthy controls to investigate whether single nucleotide polymorphisms (SNPs) in the OX40L gene are associated with sporadic breast cancer susceptibility and progression in Chinese Han women. Seven SNPs of OX40L (rs6661173, rs1234313, rs3850641, rs1234315, rs12039904, rs844648 and rs10912580) were genotyped with the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The results indicated that rs3850641G allele could increase the susceptibility to breast cancer (P = 0.009662), even in the validation study (P = 0.000151). A significant association between rs3850641 and breast cancer risk was observed under the additive model and dominant model (P = 0.01042 and 0.01942, respectively). The haplotype analysis showed that haplotype A<sub>r</sub>S844648A<sub>s</sub>10912580 was significantly associated with breast cancer, even after 10,000 permutations for haplotypes in block only (P = 0.0003). In clinicopathologic features analysis, the association between rs1234315 and C-erbB2 status was significant (P = 0.02541). Our data primarily indicates that rs3850641 of OX40L gene contributes to sporadic breast carcinogenesis in a northeast Chinese Han population.
polymorphisms of OX40L gene are associated with sporadic breast cancer in northeast Chinese Han population.

Results

Frequencies of Alleles and Genotypes between Cases and Controls

The clinical features of cases with breast cancer are summarized in Table 1. The genotype distribution of the seven SNPs was in HWE in controls \((P > 0.05)\). About 5% samples were randomly selected for direct DNA sequencing, and the reproduction rate was 100%. As shown in Table 2, a higher prevalence of rs3850641G allele was observed in breast cancer patients \((14.27\%)\) than in controls \((10.69\%)\), which showed a statistically significant association between rs3850641G allele and breast cancer risk \((P = 0.009, OR = 1.391, 95\% CI = 1.083–1.787)\) calculated by the Chi-square test with Plink 1.07 software. In the validation study, the association remained significant between rs3850641G allele and breast cancer risk \((P = 0.0001515, OR = 1.63, 95\% CI = 1.264–2.102)\), even after correction with 10,000 permutations for single markers only \((P = 0.0000318)\). However, we failed to replicate this result in the validation cohort \((P = 0.1997, OR = 1.123, 95\% CI = 0.940–1.342)\). No significant differences were found between the other SNPs and breast cancer risk.

The association analysis between genetic models and breast cancer risk demonstrated a moderate association between rs3850641G and breast cancer risk under the additive model \((P = 0.01042)\) and dominant model \((P = 0.01942)\) using a logistic regression analysis with Plink 1.07 software. The association was replicated in the validation study under the additive model \((P = 0.0001515)\) and dominant model \((P = 0.0000596)\) (Table 3). Although a moderate association was demonstrated between rs844648 and breast cancer risk under the additive model \((P = 0.0382)\) and dominant model \((P = 0.0347)\) in the study cohort, this result failed to be confirmed in the validation cohort (Table 3).

Frequencies of Haplotypes between Cases and Controls

We further analyzed the association between haplotypes and breast cancer risk. With the method described in the statistical analysis section, three blocks were identified using the Haploview program 4.1 based on the Solid Spine of LD method. As shown in Fig. S1, rs6661173 and rs1234313 \((D' = 0.87)\) belonged to the LD block 1, and they constructed three haplotypes \((\text{Grs6661173}_\text{Grs1234313}_\text{Grs12039904}, \text{Grs6661173}_\text{Grs1234313}_\text{Grs12039904} \text{and Grs6661173}_\text{Grs1234313}_\text{Grs12039904})\), which were not associated with breast cancer risk \((P > 0.05, \text{Table 4})\). Rs3850641Grs1234315Grs12039904 was less frequent appeared \((25.1\%)\) in cases and 27.0\% in controls). The haplotype \((\text{Grs3850641}_\text{Grs1234315}_\text{Grs12039904})\) had a higher frequency in cases \((45.9\%)\) than in controls \((4.9\%)\) in the study cohort \((P = 0.00001515, OR = 1.63, 95\% CI = 1.264–2.102)\), even after correction with 10,000 permutations for single markers only \((P = 0.0000318)\). However, we failed to replicate this result in the validation cohort \((P = 0.1997, OR = 1.123, 95\% CI = 0.940–1.342)\). No significant differences were found between the other SNPs and breast cancer risk.

As shown in Table 1, in the clinicopathologic features of 557 breast cancer patients are summarized, including histological grade, tumor size, lymph node metastasis and the status of estrogen receptor (ER), progesterone receptor (PR), epidermal growth factor receptor 2 (C-erbB2) and tumor protein 53 (P53) which were investigated in this study. As shown in Table S1, significant association was observed between rs1234315 and C-erbB2 status \((P_{\text{dominant}} = 0.008199, P_{\text{additive}} = 0.023896)\). Moderate association was observed between rs3850641 and tumor size \((P_{\text{additive}} = 0.04853)\) as well as between rs844648 and histological grade \((P_{\text{global}} = 0.022, \text{data not shown})\). However, no statistical association was found in

| Clinicalpathologic features | Case n (%) |
|---------------------------|------------|
| Tumor type                |            |
| IDC                       | 451 (80.97) |
| ILC                       | 13 (2.33)  |
| Intraductal carcinoma     | 41 (7.36)  |
| Mucinous adenocarcinoma   | 9 (1.62)   |
| Others                    | 43 (7.72)  |
| Tumor size                |            |
| with the diameter less than 2 cm | 185 (33.21) |
| with the diameter 2 to 5 cm | 243 (43.63) |
| with the diameter more than 5 cm | 26 (4.67)  |
| Unknown                   | 103 (18.49)|
| LN involvement            |            |
| Positive                  | 232 (41.65)|
| Negative                  | 307 (55.12)|
| Unknown                   | 18 (3.23)  |
| ER                        |            |
| Positive                  | 277 (49.73)|
| Negative                  | 199 (35.73)|
| Unknown                   | 81 (14.54)|
| PR                        |            |
| Positive                  | 342 (61.40)|
| Negative                  | 132 (23.70)|
| Unknown                   | 83 (14.90)|
| PS3                       |            |
| Positive                  | 145 (26.03)|
| Negative                  | 313 (56.19)|
| Unknown                   | 99 (17.78)|
| C-erbB-2                  |            |
| Positive                  | 185 (33.21)|
| Negative                  | 286 (51.35)|
| Unknown                   | 86 (15.44)|

Abbreviations: IDC = infiltrative ductal carcinoma; ILC = infiltrative lobular carcinoma; LN = lymph node; T2 = tumor size; ER = estrogen receptor; PR = progesterone receptor; P53 = tumor protein 53; C-erbB2 = human epidermal growth factor receptor 2. (P<0.05).
other clinicopathologic features (lymph node metastasis and the status of ER, PR and P53).

We further analyzed the association between haplotypes and clinicopathologic features. The frequency of Ars3850641Crs1234315Crs12039904 haplotype in the LD block 2 was higher in C-erbB2 positive cases ($P = 0.0245$) and Grs844648Grs10912580 haplotype in the LD block 3 had a lower frequency in C-erbB2 positive cases ($P = 0.0362$, Table S2). No significant association was observed in other clinicopathologic features.

Table 2. Alleles of the seven SNPs of OX40L gene between cases and controls.

| SNP          | Minor allele | Study cohort | Validation cohort |
|--------------|--------------|--------------|------------------|
|              |              | Cases$n = 557$ | Controls$n = 580$ | $P$ value | OR(95%CI) | Cases$n = 507$ | Controls$n = 492$ | $P$ value | OR(95%CI) |
| rs6661173    | A            | 0.06463      | 0.05776        | 0.494     | 1.127(0.7996–1.589) |
| rs1234313    | G            | 0.3402       | 0.3466         | 0.7504    | 0.9723(0.8177–1.156) |
| rs3850641    | G            | 0.1427       | 0.1069         | 0.009662  | 1.391(1.083–1.787) |
| rs1234315    | T            | 0.5144       | 0.4802         | 0.1031    | 1.147(0.9277–1.352) |
| rs12039904   | T            | 0.2756       | 0.2948         | 0.3097    | 0.9099(0.7583–1.092) |
| rs844648     | A            | 0.4722       | 0.4284         | 0.03616   | 1.193(1.011–1.408) |
| rs10912580   | G            | 0.281        | 0.2914         | 0.583     | 0.9503(0.7922–1.14) |

SNP, single nucleotide polymorphism. Allele data and basic allelic Chi-square test were analyzed using Plink 1.07. Asymptotic $P$ value, estimated odds ratio (OR) and 95% confidence interval (CI) were calculated. Significant values ($P$, $<0.05$) are in bold.

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Table 3. Genotyping and genetic models of OX40L gene SNPs in cases and controls.

| SNP          | Genetic Models | Study cohort | Validation cohort |
|--------------|----------------|--------------|------------------|
|              |                | Cases$n = 557$ | Controls$n = 580$ | $P$ value | OR (95%CI) | Cases$n = 507$ | Controls$n = 492$ | $P$ value | OR (95%CI) |
| rs6661173    | additive       | 1/70/486     | 4/59/517        | 0.4967    | 1.1260(0.8–1.584) |
|              | dominant       | 71/486       | 63/517          | 0.3249    | 1.1990(0.8355–1.72) |
|              | recessive      | 1/556        | 4/576           | 0.2276    | 0.259(0.02886–2.324) |
| rs1234313    | additive       | 62/255/240   | 68/266/246      | 0.7485    | 0.9718(0.8162–1.157) |
|              | dominant       | 317/240      | 334/246         | 0.8183    | 0.9728(0.7691–1.231) |
|              | recessive      | 62/495       | 68/512          | 0.7534    | 0.943(0.6542–1.359) |
| rs3850641    | additive       | 13/133/411   | 6/112/462       | 0.01042   | 1.387(1.08–1.782) |
|              | dominant       | 146/411      | 118/462         | 0.01942   | 1.391(1.055–1.834) |
|              | recessive      | 13/544       | 6/574           | 0.09628   | 2.2860(0.8628–6.057) |
| rs1234315    | additive       | 153/267/137  | 132/293/155     | 0.1059    | 1.1440(0.9718–1.348) |
|              | dominant       | 420/137      | 425/155         | 0.4117    | 1.1180(0.8565–1.459) |
|              | recessive      | 153/404      | 132/448         | 0.06728   | 1.2850(0.9823–1.682) |
| rs12039904   | additive       | 36/235/286   | 54/234/292      | 0.3072    | 0.9089(0.7566–1.092) |
|              | dominant       | 271/286      | 288/292         | 0.7356    | 0.9607(0.7613–1.212) |
|              | recessive      | 36/521       | 54/526          | 0.07697   | 0.6731(0.434–1.044) |
| rs844648     | additive       | 124/278/155  | 112/273/195     | 0.03836   | 1.189(1.009–1.402) |
|              | dominant       | 402/155      | 385/195         | 0.03549   | 1.314(1.02–1.692) |
|              | recessive      | 124/433      | 112/468         | 0.2202    | 1.1970(0.8981–1.594) |
| rs10912580   | additive       | 40/233/284   | 53/232/295      | 0.5829    | 0.9503(0.7921–1.14) |
|              | dominant       | 273/284      | 285/295         | 0.9663    | 0.9950(0.7885–1.256) |
|              | recessive      | 40/517       | 53/527          | 0.2298    | 0.7693(0.5014–1.18) |

SNP, single nucleotide polymorphism. *The number of cases in study cohort was 557, the number of controls in study cohort was 580, the number of cases in validation cohort was 507, and, the number of controls in validation cohort was 492. The $P$ value, odds ratio (OR) and 95% confidence interval (CI) in each comparison were assessed under an additive model (additive effect of having one additional copy of a allele, a was for the minor allele and A was for the major allele), dominant model (aa+Aa vs. AA), and recessive model (aa vs. Aa+AA) using logistic regression adjusted for age with Plink 1.07. Significant values ($P$, $<0.05$) are in bold.

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Discussion

Breast cancer is a highly heterogeneous malignancy with complex genetic patterns. Further understanding of the patient’s genetic background is critical for improving the ability to assess breast cancer and optimizing the approaches for prevention and treatment.

OX40L is an important member of the tumor necrosis factor superfamily. Costimulatory molecules play crucial roles in inflammation and anitumor immune responses. The Cross-linking of OX40L with OX40 provides a T-cell costimulatory signal, resulting in increased proliferation and cytokine production [3,21]. Recent study suggested that OX40L could regulate the balance of Th cell polarization in different cytokine microenvironments [22]. And their antitumor effects are quite variable depending on the tumor microenvironments [13,23]. Previous studies have shown that OX40L gene polymorphisms are associated with diverse autoimmune diseases. However, the association between SNPs of OX40L and breast cancer risk remain unknown. Therefore, we investigated the associations of OX40L SNPs with breast cancer risk.

In haplotype analysis, we found that haplotype Grs3850641Trs1234315Crs12039904 containing rs3850641G was associated with an increased risk of breast cancer, whereas haplotype Grs844648Ars10912580 may play a role in estrogen-related pathways and play an important role in gender-specific disease, such as breast cancer.

Previous studies showed that rs844648 and rs12039904 were implicated in susceptibility to SSc [15] and SLE [18]. These two SNPs are located in the promoter region of OX40L gene where might effect the transcriptional efficiency. In our study, we found that the rs844648A allele was associated with an increased risk of breast cancer, and the association was significant under the additive and dominant model of rs3850641 showed association with breast cancer risk. A recent report by Smirnov and colleagues confirmed that there is a highly conserved binding site of miR-125b in the 3¢ end of OX40L. Ataxia telangiectasia mutated (ATM) gene regulates OX40L expression through miR-125b implicated in breast cancer and heart disease [32]. However, in the functional study, rs3850641 did not influence the binding of nuclear protein and the OX40L expression [24], which indicated that other molecular mechanisms may be involved in the SNP functions. Taken together, rs3850641 may contribute to the regulation of hormone-related pathways and play an important role in gender-specific disease, such as breast cancer.

Recent study in GWAS demonstrated that different autoimmune diseases shared limited genetic overlap [33]. According to our results, we found that the direction of the observed associations were in agreement with previous findings in studies of cardiovascular disease, not in autoimmune diseases such as SLE. It suggested that the pathogenesis may be similar between of cardiovascular disease and breast cancer, and the association was significant under the additive and dominant models. However, this genetic association was not confirmed in our validation cohort. So, confirming this result with enlarged sample size was needed.

In haplotype analysis, we found that haplotype Grs3850641Trs1234315Crs12039904 containing rs3850641G was associated with an increased risk of breast cancer. And haplotypes Grs844648Ars10912580 and Grs844648Arsgrs10912580 may be risk factors in breast cancer, whereas haplotype Grs844648Grsgrs10912580 may play a role in estrogen-related pathways.
protective role in breast cancer. These results also suggested that rs3050641 may play an important role in the pathogenesis of breast cancer.

The clinicopathologic features analysis indicated that there are significant associations between rs12934115 and C-erbB2 status, as well as between the haplotypes A_{rs12039904}G_{rs1234312}C_{rs12039904} and G_{rs1244646}G_{rs10912580} and C-erbB2 status. C-erbB2 is an important factor in predicting the long-term survival of breast cancer patients. Overexpression of C-erbB2 leads to the activation of downstream signaling pathway associated with cell proliferation, differentiation and angiogenesis [35], which is associated with increased disease recurrence and worse prognosis [36]. This result may be correlated with the report that accumulating of Tregs at the tumor site is associated with poor prognosis [37,38]. Altogether, rs12934115 may be important for the prognosis or prediction of breast cancer.

Conclusion

Our primary data first demonstrates the genetic association of polymorphisms in OX40L gene with sporadic breast cancer. It suggests that rs3850641G is associated with an increased risk of breast cancer. OX40L gene polymorphisms may affect breast cancer risk and prognosis in Chinese Han population, northeast of China. It is necessary to confirm this result with enlarged sample size in multiethnic groups in the future.

Materials and Methods

Ethics Statement

The study is in compliance with the Helsinki declaration, and has been approved by the Medical Ethical Committee of Harbin Medical University. The informed written consent was obtained from all subjects.

Subjects

In this study, we analyzed a total of 557 female sporadic breast cancer cases and 580 age- and sex-matched healthy controls. All subjects were recruited from the Department of Breast Surgery in the Third Affiliated Hospital of Harbin Medical University. All patients (mean age at 49.1±9.9 years) were pathologically confirmed, which pathological and clinical information were obtained from medical files (Table 1). The controls were frequency-matched to cases by age (mean age at 48.3±10.1 years) and without any history of personal or familial malignancy or autoimmune disorders. The validation cohort consisted 507 breast cancer cases and 580 age- and sex-matched healthy controls. All subjects were recruited from the Department of Breast Surgery in the Third Affiliated Hospital of Harbin Medical University. All patients (mean age at 49.1±9.9 years) were pathologically confirmed, which pathological and clinical information were obtained from medical files (Table 1). The controls were frequency-matched to cases by age (mean age at 48.3±10.1 years) and without any history of personal or familial malignancy or autoimmune disorders. The validation cohort consisted 507 breast cancer cases (mean age at 49.4±10.1 years) and 492 age- and sex-matched healthy controls (mean age at 48.7±10.4 years), and the selection criteria for cases and controls was as described above. Both breast cancer cases and healthy controls were hereditarily unrelated and were recruited from Heilongjiang Province, northeast of China.

SNP Selection and Genotyping

To determine the association between SNPs of OX40L and breast cancer risk, we selected SNP loci based on the published reports, in which these SNPs were significantly associated with autoimmune diseases and heart diseases or showed gender-specific effects in some studies. Then, we selected common and potentially functional SNPs with minor allele frequency (MAF) >0.10 located in the OX40L gene using NCBI dbSNP database. Finally, we discarded the SNPs which were in high linkage disequilibrium (LD) with each other in CHB population using HapMap database. Seven SNPs (rs6061173, rs1234313, rs3850641, rs1234315, and rs12039904, rs844646, rs10912580) were selected which can be tested by PCR-RFLP method. Genomic DNA was extracted from whole blood with the universal genomic DNA Extraction Kit VER.3.0 (TaKaRa, Japan). Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The polymorphic region was amplified by PCR using a T-Gradient Thermoblock PCR System (Biometra, Germany) in a 25ul reaction solution containing 0.3ug genomic DNA (100ng/ul), 2.5ul 10× PCR buffer (Mg²⁺ plus) (TaKaRa, Japan), 2.0ul dNTPs mixture (TaKaRa, Japan), 0.25ul TaqDNA polymerase (5U/ul) (TaKaRa, Japan) and 0.1ul of each primer (10umol/L) (Invitrogen, China). Primers sequences of each SNP were listed in Table S3. The PCR products were digested with restriction enzymes (NEB, UK) according to the manufacturer’s instruction and analyzed by agarose gel electrophoresis. The accuracy of genotyping results were confirmed by direct sequencing in random samples.

Statistical Analysis

Genotype frequencies of seven SNPs were tested for Hardy–Weinberg equilibrium (HWE), which was tested using the Chi-square test. Genotype frequencies were estimated by direct counting. The basic association test for a disease trait based on comparing allele frequencies between cases and controls was achieved by Plink 1.07 software [http://pngu.mgh.harvard.edu/~purcell/plink/]. The asymptotic P-values are available. In addition to the allelic test of association, P values, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression analysis adjusted for age, assuming an additive model (additive effect of having one additional copy of a allele, a was for the minor allele and A was for the major allele), dominant model (aa vs. AA) and recessive model (aa vs. Aa+AA) with Plink 1.07 software. Linkage disequilibrium, haplotype tests and multiple testing were analyzed with the Haplovie 4.1 software [http://www.broad.mit.edu/mpg/haplovie/], which constructs haplotypes based on the D' values generating from our own data as the Solid Spine of LD method. Chi-square test was also performed to analyze the association between the SNPs and various clinical features of breast cancer with the Haplovie 4.1 software. For accurate multiple testing correction, P values of the alleles and haplotypes were permuted 10,000 times using Haplovie 4.1 software. All statistical tests were two-sided, and P values less than 0.05 were considered statistically significant.

Supporting Information

Figure S1  The pairwise D' and Haplotype-block of the seven SNPs in OX40L gene. Linkage disequilibrium (LD) strength was shown in the diamonds represented by D’ value, and bright red represent high-pairwise D’ value, which were generated by Haplovie 4.1. Blocks were defined as the method of solid spine of LD according to the values of D’ generating from our own data.

Table S1 Significant associations between OX40L SNPs and C-erbB2 status in cases. SNP, single nucleotide polymorphism. C-erbB2, human epidermal growth factor receptor 2. The number of cases with negative C-erbB2 was 286, and the number of controls with positive C-erbB2 was 185. The P values were assessed under an additive model (additive effect of having one additional copy of a allele, a was for the minor allele and A was for the major allele), dominant model (aa vs. AA) and recessive model (aa vs. Aa+AA) using logistic regression adjusted for age with Plink 1.07. Significant values (P<0.05) are in bold.
Table S2 Associations between haplotype of OX40L gene SNPs and C-erbB2 status in cases. Blocks were constructed as the method of solid spine of LD according to the values of D' generating from our own data. Haplotype data was analyzed using Haploview 4.1. Significant values (P<0.05) are in bold. (DOC)

Table S3 Information of primers and products. (DOC)

Author Contributions
Conceived and designed the experiments: LD PD. Performed the experiments: YW LD CS XF FZ. Analyzed the data: XL CS. Contributed reagents/materials/analysis tools: CY LY. Wrote the paper: YW LD.

References
1. Stuber E, Neurath M, Calderhead D, Fell HP, Strober W (1995) Cross-linking of OX40 ligand, a member of the TNF/NGF cytokine family, induces proliferation and differentiation in murine splenic B cells. Immunity 2: 507–521.
2. Murata K, Ishii N, Takano H, Miura S, Ndlhlovu LC, et al. (2000) Impairment of antigen-presenting cell function in mice lacking expression of OX40 ligand. J Exp Med 191: 563–574.
3. Oshihama Y, Tanaka Y, Tozawa H, Takahashi Y, Maliszewski C, et al. (1997) Expression and function of OX40 ligand on human dendritic cells. J Immunol 159: 5336–5340.
4. Kashivakura J, Yokoi H, Saito H, Okayama M (2004) T cell proliferation by direct cross-talk between OX40 ligand on human mast cells and OX40 on human T cells: comparison of gene expression profiles between human tonnalar and lung-cultured mast cells. J Immunol 173: 5247–5257.
5. Imura A, Hori T, Imada K, Ishikawa T, Tanaka Y, et al. (1996) The human OX40/Gp34 system directly mediates adhesion of activated T cells to vascular endothelial cells. J Exp Med 183: 2185–2195.
6. Ito T, Wang YH, Duramad O, Hanabuchi S, Perng OA, et al. (2006) OX40 ligation shuts down IL-10-producing regulatory T cells. Proc Natl Acad Sci U S A 103: 13136–13141.
7. Duan W, So T, Croft M (2008) Antagonism of airway tolerance by endotoxin/lipo polysaccharide through promoting OX40L and suppressing antigen-specific Foxp3+ T regulatory cells. J Immunol 180: 9650–9659.
8. Weinberg AD, Rivera MM, Pe'er R, Morris A, Ramstad T, et al. (2000) Engagement of the OX-40 receptor in vivo enhances antitumor immunity. J Immunol 164: 2160–2169.
9. Girevniks SI, Greten FR, Karim M (2010) Immunity, inflammation, and cancer. Cell 140: 883–899.
10. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646–674.
11. Coughlin SS, Elouene DU (2009) Breast cancer as a global health concern. Cancer Epidemiol Biomarkers Prev 18: 280–293.
12. Ali SA, Ahmad M, Lynam J, McLean CS, Entwisle C, et al. (2004) Anti-tumour therapeutic efficacy of OX40L in murine tumour model. Vaccine 22: 3583–3594.
13. Pedroza-Gonzalez A, Xie K, Wu TC, Aspord C, Tindle S, et al. (2011) Thymic stromal lymphopoietin fosters human breast tumor growth by promoting type 2 inflammation. J Exp Med 208: 479–490.
14. Cunningham Graham DS, Graham RR, Manku H, Wong AK, Whitaker JC, et al. (2000) Polymorphism at the TNF superfamily gene TNFSF4 confers susceptibility to systemic lupus erythematosus. Nat Genet 7: e1002406.
15. Gough P, Arnett FC, Tan FK, Assadi S, Dweck D, et al. (2010) Association of TNFSF4 (OX40L) polymorphisms with susceptibility to systemic sclerosis. Ann Rheum Dis 69: 530–535.
16. Wang X, Ria M, Kelhormson PM, Erikson P, Higgs DG, et al. (2003) Positional identification of TNFSF4, encoding OX40 ligand, as a gene that influences atherosclerosis susceptibility. Nat Genet 37: 563–572.
17. Rossin-Castillo L, Broen JC, Simeon CP, Beretta L, Vouk MC, et al. (2011) A replication study confirms the association of TNFSF4 (OX40L) polymorphisms with systemic sclerosis in a large European cohort. Ann Rheum Dis 70: 630–641.
18. Chang YK, Yang W, Zhao M, Mok CC, Chan TM, et al. (2009) Association of BANK1 and TNFSF4 with systemic lupus erythematosus in Hong Kong Chinese. Genes Immun 10: 414–420.
19. Delgado-Vega AM, Abelson AK, Sanchez E, Witte T, D’Alfonso S, et al. (2009) Replication of the TNFSF4 (OX40L) promoter region association with systemic lupus erythematosus. Genes Immun 10: 248–253.
20. Masihino Y, Suzuki Y, Hatori K, Tabara Y, Miki T, et al. (2008) Association of TNFRSF4 gene polymorphisms with essential hypertension. J Hypertens 26: 902–913.
21. Stuber E, Strober W (1996) The T cell-B cell interaction via OX40-OX40L is necessary for the T cell-dependent humoral immune response. J Exp Med 183: 979–989.
22. Liu YJ, Soumelis V, Watanabe N, Ito T, Wang YH, et al. (2007) TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. Annu Rev Immunol 25: 193–219.
23. Morris A, Vetto JT, Ramstad T, Fumatake C, Chooulun E, et al. (2001) Induction of anti-mammary cancer immunity by engaging the OX-40 receptor in vivo. Breast Cancer Res Treat 67: 71–80.
24. Ria M, Lagercrantz J, Sunnegard A, Boegst S, Hamsten A, et al. (2011) A common polymorphism in the promoter region of the TNFSF4 gene is associated with lower allele-specific expression and risk of myocardial infarction. PLoS One 6: e17632.
25. Yamazaki S, Yamada Y, Metoki N, Yoshiha H, Satoh K, et al. (2006) Genetic risk for atherothrombotic cerebral infarction in individuals stratified by sex or conventional risk factors for atherosclerosis. Int J Mol Med 18: 871–873.
26. Malarstig A, Eriksson P, Rose L, Deld RA, Hamsten A, et al. (2006) Genetic variants of tumor necrosis factor superfamily, member 4 (TNFSF4), and risk of incident atherothrombosis and venous thromboembolism. Clin Chem 54: 833–840.
27. Rots EN (2000) The X-tiles in immunity: sex-based differences predispose immune responses. Nat Rev Immunol 8: 737–744.
28. Lin PY, Sun L, Thiébaud SR, Ludwig SM, Vadlamudi RK, et al. (2010) Bs1-h1-dependent sex-related differences in tumor immunity and immunotherapy responses. J Immunol 185: 2747–2753.
29. Takeda I, Ine S, Källén N, Ndlhlovu LC, Murata K, et al. (2004) Distinct roles for the OX40-OX40 ligand interaction in regulatory and nonregulatory T cells. J Immunol 179: 3590–3599.
30. Vu MD, Xiao X, Gao W, Degauque N, Chen M, et al. (2007) OX40 agonist therapy enhances CD8+ infiltrate and decreases immune suppression in the tumor. Cancer Res 67: 5206–5215.
31. Smirnov DA, Cheung VG (2008) ATM gene mutations result in both recessive and dominant expression phenotypes of genes and microRNAs. Am J Hum Genet 83: 243–253.
32. Ramos PS, Criswell LA, Moorer KL, Ciemiea ME, Williams AH, et al. (2011) A comprehensive analysis of shared loci between systemic lupus erythematosus (SLE) and sixteen autoimmune diseases reveals limited genetic overlap. PLoS Genet 7: e1002406.
33. Shiaklas PC, Singh KK, Quan A, Al-Oumama M, Tseoh H, et al. (2011) BRCA1 is an essential regulator of heart function and survival following myocardial infarction. Nat Commun 2: 593.
34. Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2: 127–137.
35. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, et al. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 235: 177–182.
36. Rakha EA, El-Sayed ME, Green AR, Paish EC, Powe DG, et al. (2007) OX40 agonist therapy enhances CD8+ infiltration and decreases immune suppression in the tumor. Cancer Res 67: 5206–5215.
37. Soerjomataram I, Louwman MW, Ribot JG, Roukema JA, Coebergh JW (2008) An overview of prognostic factors for long-term survivors of breast cancer. Breast Cancer Res Treat 107: 309–330.