High Prevalence of the BIM Deletion Polymorphism in Young Female Breast Cancer in an East Asian Country

Ching-Hung Lin1,2, Chen-Yang Shen3,4,5, Jih-Hsiang Lee3, Chiun-Sheng Huang6 Chih-Hsin Yang1,7, Wen-Hung Kuo6, Dwan-Ying Chang1, Chia-Ni Hsiung4, Kuan-Ting Kuo6, Wei-Wu Chen1, I-Chun Chen1, Pei-Fang Wu1, Sung-Hsin Kuo1,2, Chien-Jen Chen9, Yen-Shen Lu1,2, Ann-Lii Cheng1,2,7* 1 Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan, 2 Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan, 3 Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan, 4 Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, 5 School of Public Health, China Medical University, Taichung, Taiwan, 6 Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan, 7 Graduate Institute of Oncology and Cancer Research Centre, College of Medicine, National Taiwan University, Taipei, Taiwan, 8 Department of Pathology, National Taiwan University Hospital, Taipei, Taiwan, 9 Genomics Research Center, Academia Sinica, Taipei, Taiwan

☯ These authors contributed equally to this work.

* alcheng@ntu.edu.tw

Abstract

Background

A rapid surge of female breast cancer has been observed in young women in several East Asian countries. The BIM deletion polymorphism, which confers cell resistance to apoptosis, was recently found exclusively in East Asian people with prevalence rate of 12%. We aimed to evaluate the possible role of this genetic alteration in carcinogenesis of breast cancer in East Asians.

Method

Female healthy volunteers (n = 307), patients in one consecutive stage I-III breast cancer cohort (n = 692) and one metastatic breast cancer cohort (n = 189) were evaluated. BIM wild-type and deletion alleles were separately genotyped in genomic DNAs.

Results

Both cancer cohorts consistently showed inverse associations between the BIM deletion polymorphism and patient age (≤35 y vs. 36-50 y vs. >50 y: 29% vs. 22% vs. 15%, P = 0.006 in the consecutive cohort, and 40% vs. 23% vs. 13%, P = 0.023 in the metastatic cohort). In healthy volunteers, the frequencies of the BIM deletion polymorphism were similar (13%-14%) in all age groups. Further analyses indicated that the BIM deletion polymorphism was not associated with specific clinicopathologic features, but it was associated with poor overall survival (adjusted hazard ratio 1.71) in the consecutive cohort.
Conclusions

*BIM* deletion polymorphism may be involved in the tumorigenesis of the early-onset breast cancer among East Asians.

Introduction

The incidence of breast cancer among Asian women is in general lower than that in Western countries. However, all health statistics indicated that breast cancer has been rapidly increasing in recent decades in East Asia, including Singapore, Korea, Japan, and Taiwan [1–4]. Compared to Caucasian American women, the age-period-cohort analyses consistently revealed a much stronger birth cohort effect on the breast cancer incidence of Singaporean, Japanese, and Taiwanese women [1,3,4]. This strong birth cohort effect correlated directly with a rapid increase in the incidence of early-onset breast cancer in these countries. Intuitively, Westernized lifestyle is thought to be the major cause of this rapidly increasing young female breast cancer (YFBC) in Asia [5]. However, our recent study demonstrated a major discrepancy of molecular subtype distributions between Taiwanese and Caucasian YFBC. In contrast to their Western counterpart, Taiwanese YFBCs are characterized by a luminal A subtype (defined as estrogen receptor [ER] and/or progesterone receptor [PR] positive and human epidermal growth factor receptor 2 [HER2] negative) prevalence, and low basal-like subtype prevalence [6]. These findings implied that the emerging YFBCs in Taiwan might not just be a mirror image of their Western counterparts. We hypothesize that some unique genetic factors or interactions between genetic factors and environmental factors may play a role in East Asian YFBC carcinogenesis.

Recently, Ng KP et al. discovered a common germline polymorphism (deletion of intron 2 of *BIM* gene) which was uniquely detected in East-Asian people (12.3% carrier frequency) and was absent in Africans and Caucasians. *BIM* deletion polymorphism conferred an inferior response to tyrosine kinase inhibitors in patients with chronic myeloid leukemia and epidermal growth factor receptor mutated non-small cell lung cancer [7–9]. The *BIM* gene encodes B-cell lymphoma 2 interacting mediator of cell death (BIM) protein, which is a member of the Bcl-2 family. Wild type BIM protein, which contains a BCL2-homology domain 3 (BH3), which functions as an apoptosis facilitator and has been shown to mediate apoptosis in response to stimuli such as cytokine deprivation, deregulated calcium flux and microtubule perturbation. Thus, BIM is considered a protector of tissue homeostasis [10,11]. The *BIM* deletion polymorphism switched *BIM* splicing from exon 4 to exon 3, and resulted in expression of BIM isoforms lacking the pro-apoptotic BH3 domain and conferred intrinsic resistance to tyrosine kinase inhibitors [7].

Since *BIM* deletion polymorphism is unique in East Asian people, and its product (BIM isoforms) may be linked to tumorigenesis, it is crucial to clarify whether this genetic change plays a role in the carcinogenesis of YFBC in East Asian women.

Materials and Methods

Patients and sample collection

All participants in this study gave written informed consent. The study received approval from the National Taiwan University Hospital (NTUH) ethics committee (201307001RINA). The study included 307 female healthy volunteers, 692 patients with stage I-III breast cancer in one
consecutive cohort and 189 patients with ER+/HER2- breast cancer in one metastatic cohort with available germline or tumor DNAs (S1–S3 Datasets). The healthy volunteers participated in the prior study exploring the association of breast cancer and gene polymorphism [12]. The consecutive cancer cohort was obtained from a prospectively collected database which included stage I-III breast cancer consecutively newly diagnosed at NTUH between 2004 and 2006 [6]. The metastatic cancer cohort was obtained from a retrospectively collected database which includes patients with ER+/HER2- metastatic breast cancer patients diagnosed at NTUH between 2001 and 2006. To avoid bias by double counting, we excluded 19 patients from the consecutive cohort because these patients were included in the consecutive cohort and had distant metastasis between 2004 and 2006. The methods and definitions of ER and HER2 positivity were previously described [6]. For ER and PR, Tumors with \( \geq 10\% \) positively-staining nuclei were considered positive. The HER2 status was considered positive if score 3+ by immunohistochemical analysis or 2+ with gene amplification on fluorescence in situ hybridization.

Evaluation of BIM deletion polymorphism

The DNAs from healthy volunteers were extracted from blood specimens. The DNAs from patients in consecutive and metastatic cancer cohorts were extracted from formalin-fixed paraffin-embedded tumor specimens. The genomic DNAs of blood and tumor specimens were isolated using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA, USA). For DNAs from each blood or tumor specimen, we performed two separate polymerase chain reaction reactions to determine the presence of the wild-type and deletion alleles as previously described [7]. The forward and reverse primers for the deletion allele were CCACCAATGGAAAAGTTCA and GGCACAGCCTCTATGGAGAA, respectively. The forward and reverse primers for the wild-type allele were CCACCAATGGAAAAGGTTCA and CTGTCATTTCTCCCCACCAC, respectively. The resulting PCR products from the deletion (1,323 bp) and the wild-type (4,226 bp) alleles were analyzed on 1% agarose gels. For each PCR, we used genomic DNAs from KCL22 and PC-9 cells as positive and negative control, respectively.

Statistical analysis

Data on clinicopathological features between wild and deleted BIM groups were compared using chi-square test (or two tailed Fisher’s exact test if expected number of each cell was less than five cases). The Mantel-Haenszel chi-square test was used to verify age, histologic grade, tumor size, lymph node status and American Joint Committee on Cancer (AJCC) stage-related trend. For survival analysis in the consecutive cancer cohort, only patients with stage I-III breast cancer were included, and distant metastasis free survival (DMFS) and overall survival (OS) were used as the endpoints. DMFS was defined as the duration from diagnosis to confirmation of distant recurrences. OS was defined as the duration from breast cancer diagnosis to death from any cause. Survival curves were constructed using the Kaplan-Meier method. The associations between each of the categorical variables and survival were analyzed using the log-rank test. Cox proportional hazards analysis was used to determine the relative contribution of various factors to survival. The backward stepwise variable selection procedure was applied to obtain the best candidate final Cox’s proportional hazards model. A \( P \) value \( \leq 0.05 \) was used to indicate statistical significance; all tests were two-tailed. All statistical analyses were performed using the statistical package SPSS for Windows (Version 17.0).
Results

Frequencies of BIM deletion polymorphism by age groups among healthy volunteers and breast cancer patients

The healthy volunteers had a median age of 48 (range 25–80) years at enrollment. The patients in consecutive and metastatic cancer cohorts had a median age of 49 (range 23–86) years and 50 (range 26–82) years at initial diagnosis of breast cancer, respectively. Among the 307 healthy volunteers, BIM deletion polymorphism was detected in 48 (14%) subjects and the frequencies were similar among the three age groups (≤35 vs. 36–50 vs. >50 years, 13% vs. 14% vs. 14%, P = 0.974). Compared with healthy volunteers, patients in the consecutive cancer cohort (19% vs. 14%, P = 0.018) had significantly higher frequencies of BIM deletion polymorphism and patients in the metastatic cancer cohort (19% vs. 14%, P = 0.089) had a trend toward higher frequencies of BIM deletion polymorphism (Table 1).

Both cancer cohorts consistently showed inverse association of BIM deletion polymorphism with patient age (≤35 years vs. 36–50 years vs. >50 years: 29% vs. 22% vs. 15%, P = 0.006 in consecutive cohort, and 40% vs. 23% vs. 13%, P = 0.023 in metastatic cohort). Among women ≤50 years, both cancer cohorts consistently showed higher frequencies of BIM deletion polymorphism than healthy volunteers (consecutive cancer cohort, 23% vs.13%, P = 0.005; metastatic cancer cohort, 25% vs. 13%, P = 0.010). In contrast, the frequencies of BIM deletion polymorphism were quite similar (healthy volunteer, 14%; consecutive cancer cohort, 15%; metastatic cancer cohort, 13%) among the three study groups of women >50 years (Table 1 and Fig 1).

Patient clinicopathological characteristics by BIM status in two breast cancer cohorts

Among the 692 patients with stage I-III breast cancer in the consecutive cohort, BIM deletion polymorphism was detected in 140 (19%) subjects. The BIM deletion polymorphism rate was significantly higher in young patients. Other clinicopathological variables including histology, histologic grade, AJCC stage, ER status, PR status, HER2 status and molecular subtype were not significantly associated with this polymorphism (Table 2).

Among the 189 patients with stage ER+/HER2- metastatic breast cancer in the metastatic cohort, BIM deletion polymorphism was detected in 36 (19%) subjects. Consistent with that in consecutive cohort, the BIM deletion polymorphism rate was significantly higher in young patients and clinicopathological variables including histology, histologic grade, PR status, recurrence status and metastatic site were not significantly associated with BIM deletion polymorphism (Table 3).

Table 1. Comparison of BIM deletion polymorphism in women benign breast disease and two breast cancer cohorts with healthy volunteers among age groups.

| BIM polymorphism | ≤35 years | 36–50 years | >50 years | Whole |
|------------------|-----------|-------------|-----------|-------|
|                   | wild | deleted | P | wild | deleted | P | wild | deleted | P | wild | deleted | P |
| Healthy (reference) | 42 (88) | 6 (13) | | 140 (86) | 22 (14) | | 125 (86) | 20 (14) | | 307 (86) | 48 (14) | |
| Consecutive cohort | 37 (71) | 15 (29) | 0.045 | 254 (78) | 71 (22) | 0.029 | 267 (85) | 48 (15) | 0.640 | 558 (81) | 134 (19) | 0.018 |
| Metastatic cohort | 9 (60) | 6 (40) | 0.028* | 62 (78) | 18 (23) | 0.079 | 82 (87) | 12 (13) | 0.820 | 153 (81) | 36 (19) | 0.089 |

* two tailed Fisher’s exact test.

doi:10.1371/journal.pone.0124908.t001
Prognostic value of \textit{BIM} deletion polymorphism in patients with stage I-III breast cancer

In consecutive cancer cohort, the median follow-up duration among the 692 patients with stage I-III breast cancer was 81.7 months (95% confidence interval (CI), 80.1–83.4). The 6-year distant metastasis-free survival rate was 92% for stage I disease, 82% for stage II disease, and 70% for stage III disease. The 6-year overall survival rate was 95% for stage I disease, 90% for stage II disease, and 74% for stage III disease.

Traditional prognostic factors such as tumor size, axillary lymph node status, ER expression, PR expression and HER2 status were associated with DMFS and/or OS in univariate and/or multivariate analyses. Univariate determined that \textit{BIM} deletion polymorphism was not associated with DMFS (hazard ratio \([HR] = 1.11, P = 0.636\)) and OS (\(HR = 1.45, P = 0.125\)). Multivariate analysis determined that \textit{BIM} deletion polymorphism was not associated with DMFS, but it was significantly associated with shorter OS (adjusted \(HR = 1.71, P = 0.027\)) (Table 4).

Discussion

High prevalence of \textit{BIM} deletion polymorphism in young Taiwanese breast cancer patients suggests that this East Asian specific genetic trait is involved in the tumorigenesis of early-onset breast cancer among East Asians. This finding supports our hypothesis that, in addition to environmental and lifestyle factors, certain genetic factors may play a role in Asian young breast cancer development.

\textit{BIM} deletion polymorphism was found exclusively in East Asian individuals (12.3% carrier frequency) \cite{7}. In our study, the frequency of the polymorphism in the whole healthy volunteers (14%) was close to that reported by Ng KP et al., and the frequencies were similar among \(\leq 35\) (13%), 36–50 (14%), and \(> 50\) (14%) age groups. In breast cancer patients \(> 50\) years, the frequencies of \textit{BIM} deletion polymorphism in both cancer cohorts were not significantly different from healthy volunteers. In contrast, among subjects \(< 50\) years, higher \textit{BIM} deletion polymorphism frequencies were consistently shown in both cancer cohorts than healthy
volunteers. Among very young (≤35 years) patients, the frequencies of BIM deletion polymorphism reached up to 29% and 40% in the consecutive and metastatic cancer cohorts, respectively.

Since no significant association between BIM deletion polymorphism and other clinico-pathological factors except age was observed, we hypothesize that BIM deletion polymorphism may mediate the cancer initiation rather than tumor progression. However, how and why this genetic change affects the young ladies in East Asians remains unclear. In addition, the major limitation of the present study is lack of comprehensive information of breast cancer risk.

### Table 2. The characteristics of patients in the consecutive cancer cohort by BIM deletion polymorphism.

| Characteristics       | No. | No. (%)                     | P value |
|-----------------------|-----|-----------------------------|---------|
| Age at initial diagnosis |    | All (n = 692) BIM wild (n = 558) BIM deleted (n = 134) |         |
| ≤35 years              | 52  | 37 (71)                     | 15 (29) | 0.006 |
| 36–50 years            | 325 | 254 (78)                    | 71 (22) |
| >50 years              | 315 | 267 (85)                    | 48 (15) |
| Histology type         |     |                            |         | 0.783 |
| Ductal carcinoma       | 659 | 532 (81)                    | 127 (19) |
| Others                 | 33  | 26 (79)                     | 7 (21)  |
| Histologic grade       |     |                            |         | 0.556 |
| I                      | 131 | 108 (82)                    | 23 (18) |
| II                     | 362 | 293 (81)                    | 69 (19) |
| III                    | 166 | 131 (79)                    | 35 (20) |
| Unknown                | 33  | 26                          | 7       |
| Tumor size             |     |                            |         | 0.186 |
| <2 cm                  | 300 | 237 (79)                    | 63 (21) |
| 2–5 cm                 | 331 | 268 (81)                    | 68 (19) |
| >5 cm                  | 61  | 53 (87)                     | 8 (13)  |
| Axillary lymph node    |     |                            |         | 0.494 |
| None or cN0            | 374 | 298 (80)                    | 76 (20) |
| 1–3 or cN1             | 194 | 160 (82)                    | 34 (18) |
| 4–9 or cN2             | 78  | 60 (77)                     | 18 (23) |
| ≥10 or cN3             | 46  | 40 (87)                     | 6 (13)  |
| AJCC stage             |     |                            |         | 0.881 |
| I                      | 237 | 191 (81)                    | 46 (19) |
| II                     | 327 | 265 (81)                    | 62 (19) |
| III                    | 128 | 102 (80)                    | 26 (20) |
| ER status              |     |                            |         | 0.434 |
| Negative               | 221 | 182 (82)                    | 39 (18) |
| Positive               | 471 | 376 (80)                    | 95 (20) |
| PR status              |     |                            |         | 0.921 |
| Negative               | 395 | 318 (81)                    | 77 (19) |
| Positive               | 297 | 240 (81)                    | 57 (19) |
| HER2 status            |     |                            |         | 0.137 |
| Negative               | 544 | 445 (82)                    | 99 (18) |
| Positive               | 148 | 113 (76)                    | 35 (24) |

AJCC, American Joint Committee on Cancer; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

doi:10.1371/journal.pone.0124908.t002
factors such as menstruation history, family history, pregnancy and birth history, alcohol consumption, and weight. To confirm \textit{BIM} deletion polymorphism as a susceptible gene for YFBC carcinogenesis in East Asia, the validation by a well designed case control study is mandatory.

\textit{BIM} is essential for initiation of various physiological apoptotic situations, including developmentally programmed cell death and stress-induced apoptosis. \textit{BIM} is considered a protector of tissue homeostasis \cite{10,11}. The breakdown of tissue homeostasis may lead to various pathological situations including tumor formation. In the mouse model of B cells and kidney epithelial cells, the loss of single \textit{BIM} sensitizes the mice to tumorigenesis \cite{13,14}. In mammary gland, disruption of the BH3-only proapoptotic factor \textit{BIM} in mice prevents induction of apoptosis in and clearing of the lumen in terminal end buds during puberty. The findings indicate that \textit{BIM} is a critical regulator of luminal space formation and maintenance during mammary morphogenesis \cite{15–17}. Since \textit{BIM} deletion polymorphism is germline genetic event and we did not observe significant association between \textit{BIM} deletion polymorphism and other clinico-pathological factors except age, we suggest that \textit{BIM} deletion polymorphism may mediate the cancer initiation rather than tumor progression in human mammary gland.

\textit{BIM} plays important roles not only in tumorigenesis but also in treatment response. Previous preclinical studies have shown that \textit{BIM} plays a key role in the anoikis, an apoptosis triggered by detachment from the extracellular matrix, of various tumor cells \cite{18–20}. Absence of wild type \textit{BIM} protein has been linked to resistance to chemotherapy and tyrosine kinase inhibitors in several cancer types \cite{14,21–30}. In breast cancer, decrease of \textit{BIM} expression has been linked to resistance to estrogen deprivation and a HER2 tyrosine kinase inhibitor \cite{31,32}. \textit{BIM} deletion polymorphism is heterozygous, so it can transcribe both wild type \textit{BIM} protein and \textit{BIM} isoforms which lack the BH3 domain. Although wild type \textit{BIM} protein from single allele may retain certain pro-apoptosis functions, the net activity of \textit{BIM} protein and isoforms in

| Characteristics | No. (n = 189) | \textit{BIM} wild (n = 153) | \textit{BIM} deleted (n = 36) | \textit{P} value |
|-----------------|---------------|----------------------------|-----------------------------|----------------|
| Age at initial diagnosis | | | | 0.023* |
| \leq 35 years | 15 | 9 (60) | 6 (40) | |
| 36–50 years | 80 | 62 (78) | 18 (23) | |
| >50 years | 94 | 82 (87) | 12 (13) | |
| Histology | | | | 0.374 |
| Ductal carcinoma | 162 | 133 (82) | 29 (18) | |
| Others | 19 | 14 (74) | 5 (26) | |
| Unknown | 8 | 6 | 2 | |
| Grade | | | | 0.583 |
| I | 27 | 22 (81) | 5 (19) | |
| II | 75 | 62 (83) | 13 (17) | |
| III | 29 | 22 (76) | 7 (24) | |
| Unknown | 58 | 47 | 11 | |
| PR Status | | | | 0.139 |
| Negative | 61 | 53 (87) | 8 (13) | |
| Positive | 126 | 98 (78) | 28 (22) | |
| Unknown | 2 | 2 | 0 | |

DFI, disease-free interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

*two tailed Fisher’s exact test.

doi:10.1371/journal.pone.0124908.t003
Table 4. Analyses of distant metastasis-free and overall survival in patients with stage I-III breast cancer.

| BIM polymorphism       | No.  | DMFS       | OS         |
|------------------------|------|------------|------------|
|                        |      | HR (95% CI)| P          | Adjusted HR (95% CI) | P          |
| Wild                   | 558  | 1.00       | 0.636      | NS                  | 0.125      |
| Deleted                | 134  | 1.11 (0.72–1.73) | 1.45 (0.90–2.33) | 1.71 (1.06–2.77) |

| Age                    |      |            |            |
|------------------------|------|------------|------------|
| ≤35 years              | 52   | 1.00       | 0.629      | NS                  | 0.008      |
| 36–50 years            | 325  | 1.13 (0.51–2.48) | 0.53 (0.24–1.16) | 0.48 (0.22–1.06) |
| >50 years              | 315  | 1.32 (0.60–2.88) | 1.08 (0.51–2.27) | 0.85 (0.40–1.82) |

| Histologic grade       |      |            |            |
|------------------------|------|------------|------------|
| I                      | 131  | 1.00       | 0.008      | NS                  | 0.002      |
| II                     | 362  | 1.82 (01.0–3.31) | 1.92 (0.90–4.10) |
| III                    | 166  | 2.80 (1.49–5.25) | 3.67 (1.70–7.93) |
| Unknown                | 33   | 2.75 (1.14–6.64) | 3.38 (1.22–9.32) |

| Tumor size             |      |            |            |
|------------------------|------|------------|------------|
| ≤ 2 cm                 | 300  | 1.00       | <0.001     | 0.022      | <0.001     |
| 2–5 cm                 | 311  | 1.81 (1.19–2.74) | 1.35 (0.87–2.09) | 2.20 (1.32–3.68) | 1.49 (0.87–2.56) |
| > 5 cm                 | 61   | 4.34 (2.55–7.38) | 2.38 (1.29–4.40) | 5.66 (3.09–10.36) | 2.69 (1.34–5.41) |

| Axillary lymph node    |      |            |            |
|------------------------|------|------------|------------|
| None or cN0            | 374  | 1.00       | <0.001     | <0.001     | <0.001     |
| 1–3 or cN1             | 194  | 2.79 (1.81–4.30) | 2.47 (1.58–3.89) | 2.39 (1.41–4.06) | 2.29 (1.31–4.01) |
| 4–9 or cN2             | 78   | 3.04 (1.76–5.26) | 2.26 (1.24–4.12) | 4.13 (2.29–7.44) | 3.26 (1.70–6.23) |
| ≥ 10 or cN3            | 46   | 4.43 (2.46–7.99) | 2.97 (1.54–5.72) | 5.39 (2.84–10.24) | 3.67 (1.78–7.57) |

| ER status              |      |            |            |
|------------------------|------|------------|------------|
| Negative               | 221  | 1.00       | 0.008      | 0.004      | <0.001     | <0.001     |
| Positive               | 471  | 0.61 (0.42–0.88) | 0.58 (0.40–0.84) | 0.41 (0.27–0.62) | 0.43 (0.28–0.67) |

| PR status              |      |            |            |
|------------------------|------|------------|------------|
| Negative               | 396  | 1.00       | 0.003      | NS         | <0.001     |
| Positive               | 296  | 0.56 (0.38–0.82) | 0.40 (0.25–0.65) |

| HER2 status            |      |            |            |
|------------------------|------|------------|------------|
| No                     | 544  | 1.00       | 0.313      | NS         | 0.509      | NS         |
| Yes                    | 148  | 1.24 (0.82–1.87) | 1.18 (0.73–1.90) |

DMFS, distant metastasis-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2

doi:10.1371/journal.pone.0124908.004
BIM deletion polymorphism cells remains inadequate in certain tumor types [7]. Our multivariate analysis showed that BIM deletion polymorphism was significantly associated with shorter OS in patients with stage I-III breast cancer (Table 4). However, it was not significantly associated with DMFS. Because of the association between BIM deletion polymorphism and age group, we conducted the stratified survival analysis in the three age groups (≤35, 36–50, and >50 years). Although the association of BIM deletion polymorphism with DMFS and OS did not reach statistical significance in the three age groups, the adjusted HRs were numerically higher in younger patients. In age group ≤35 year, the adjusted HRs were 6.03 for DMFS, and 1.99 for OS (S1 Table). Therefore, the prognostic value of BIM deletion polymorphism in patients with breast cancer warrants to be validated.

Samples used for genotyping in this study were different between healthy volunteers (blood) and cancer patients (tumor). We have analyzed both the wild and deletion alleles with positive and negative controls and did not detect homozygous deletion of BIM gene in any individual sample. In addition, both cancer cohorts consistently showed inverse association of age with BIM deletion polymorphism. Among cancer patients, women ≤50 years had significantly higher frequency of BIM deletion polymorphism than patients aged >50 years (Fig 1). Therefore, the use of different types of samples is unlikely to produce bias of our findings.

In summary, we have discovered a high prevalence of BIM deletion polymorphism in young patients with breast cancer in Taiwan, and this polymorphism may be associated with patients’ poor survival. As a potential susceptible genetic factor, BIM deletion polymorphism may interact with their contemporary environmental and lifestyle factors and contribute to the YFBC carcinogenesis in East Asians. Clarification of the underlying mechanisms and interaction between BIM deletion and their contemporary environmental factors is an important step toward mitigating the rapid surge of YFBC in East Asia.

Supporting Information

S1 Dataset. Raw data of healthy volunteers.
(XLS)

S2 Dataset. Raw data of patients in the consecutive cohort.
(XLS)

S3 Dataset. Raw data of patients in the metastatic cohort.
(XLS)

S1 Table. Stratified survival analyses of BIM deletion polymorphism by age groups in patients with stage I-III breast cancer.
(DOCX)

Acknowledgments

We thank the members of the Office of Medical Records at National Taiwan University for their help in assessing the clinical data. We thank Dr. S Tiong Ong (from Duke National University of Singapore Graduate Medical School, Singapore) for kindly providing genomic DNA of KCL22 cell line as positive control of BIM deletion polymorphism.

Author Contributions

Conceived and designed the experiments: CHL CYS CHY YSL ALC. Performed the experiments: CHL CYS CHY YSL ALC. Analyzed the data: CHL CYS JHL YSL. Contributed
reagents/materials/analysis tools: CHL CYS JHL CSH CHY WHK DYC CNH KTK WWC ICC SHK PFW CJC ALC. Wrote the paper: CHL CYS JHL YSL ALC.

References

1. Sim X, Ali RA, Wedren S, Goh DL, Tan CS, Reilly M, et al. Ethnic differences in the time trend of female breast cancer incidence: Singapore, 1968–2002. BMC Cancer. 2006; 6: 261. PMID: 17078893

2. Yoo KY, Kim Y, Park SK, Kang D. Lifestyle, genetic susceptibility and future trends of breast cancer in Korea. Asian Pac J Cancer Prev. 2006; 7: 679–682. PMID: 17250452

3. Matsuno RK, Anderson WF, Yamamoto S, Tsukuma H, Pfeiffer RM, Kobayashi K, et al. Early- and late-onset breast cancer types among women in the United States and Japan. Cancer Epidemiol Biomarkers Prev. 2007; 16: 1437–1442. PMID: 17627009

4. Shen YC, Chang CJ, Hsu C, Cheng CC, Chiu CF, Cheng AL. Significant difference in the trends of female breast cancer incidence between Taiwanese and Caucasian Americans: implications from age-period-cohort analysis. Cancer Epidemiol Biomarkers Prev. 2005; 14: 1986–1990. PMID: 16103449

5. Porter P. "Westernizing" women's risks? Breast cancer in lower-income countries. N Engl J Med. 2008; 358: 213–216. doi:10.1056/NEJMp0708307 PMID: 18199859

6. Lin CH, Liau JY, Lu YS, Huang CS, Lee WC, Kuo KT, et al. Molecular subtypes of breast cancer emerging in young women in Taiwan: evidence for more than just westernization as a reason for the disease in Asia. Cancer Epidemiol Biomarkers Prev. 2009; 18: 1807–1814. doi: 10.1158/1055-9965.EPI-09-0096 PMID: 19505913

7. Ng KP, Hillmer AM, Chuah CT, Juan WC, Ko TK, Teo AS, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med. 2012; 18: 521–528. doi: 10.1038/nm.2713 PMID: 22426421

8. Zhao M, Zhang Y, Cai W, Li J, Zou F, Cheng N, et al. The Bim deletion polymorphism clinical profile and its relation with tyrosine kinase inhibitor resistance in Chinese patients with non-small cell lung cancer. Cancer. 2014; 120: 2299–2307. doi: 10.1002/cncr.28725 PMID: 24736748

9. Isobe K, Hata Y, Tochigi N, Kaburaki K, Kobayashi H, Makino T, et al. Clinical significance of BIM deletion polymorphism in non-small-cell lung cancer with epidermal growth factor receptor mutation. J Thorac Oncol. 2014; 9: 483–487. doi: 10.1097/JTO.0000000000000125 PMID: 24736070

10. O'Connor L, Strasser A, O'Reilly LA, Hausmann G, Adams JM, Cory S, et al. Bim: a novel member of the Bcl-2 family that promotes apoptosis. EMBO J. 1998; 17: 384–395. PMID: 9430630

11. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, et al. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. Science. 1999; 286: 1735–1738. PMID: 10576740

12. Yu JC, Hsiung CN, Hsu HM, Bao BY, Chen ST, Hsu GC, et al. Genetic variation in the genome-wide predicted estrogen response element-related sequences is associated with breast cancer development. Breast Cancer Res. 2011; 13: R13. doi: 10.1186/bcr2821 PMID: 21281495

13. Egle A, Harris AW, Bouillet P, Cory S. Bim is a suppressor of Myc-induced mouse B cell leukemia. Proc Natl Acad Sci U S A. 2004; 101: 6164–6169. PMID: 15079075

14. Tan TT, Degenhardt K, Nelson DA, Beaudoin B, Nieves-Neira W, Bouillet P, et al. Key roles of BIM-driven apoptosis in epithelial tumors and rational chemotherapy. Cancer Cell. 2005; 7: 227–238. PMID: 15766661

15. Mailleux AA, Overholtzer M, Schmelzle T, Bouillet P, Strasser A, Brugge JS. BIM regulates apoptosis during mammary ductal morphogenesis, and its absence reveals alternative cellular death mechanisms. Dev Cell. 2007; 12: 221–234. PMID: 17276340

16. Whelan KA, Caldwell SA, Shahriari KS, Jackson SR, Franchetti LD, Johannes GJ, et al. Hypoxia suppression of Bim and Bmf blocks anoikis and luminal clearing during mammary morphogenesis. Mol Biol Cell. 2010; 21: 3829–3837. doi: 10.1091/mbc.E10-04-0353 PMID: 20661305

17. Reginato MJ, Mills KR, Becker EB, Lynch DK, Bonni A, Muthuswamy SK, et al. Bim regulation of lumen formation in cultured mammary epithelial acini is targeted by oncogenes. Mol Cell Biol. 2005; 25: 4591–4601. PMID: 15999862

18. Woods NT, Yamaguchi H, Lee FY, Bhatta KN, Wang HG. Anoikis, initiated by McI-1 degradation and Bim induction, is deregulated during oncogenesis. Cancer Res. 2007; 67: 10744–10752. PMID: 18006817

19. Puthalakath H, Villunger A, O'Reilly LA, Beaumont JG, Coultas L, Cheney RE, et al. Bmf: a proapoptotic BH3-only protein regulated by interaction with the myosin V actin motor complex, activated by anoikis. Science. 2001; 293: 1829–1832. PMID: 11546872
20. Reginato MJ, Mills KR, Paulus JK, Lynch DK, Debnath J, et al. Integrins and EGFR coordinately regulate the pro-apoptotic protein Bim to prevent anoikis. Nat Cell Biol. 2003; 5: 733–740. PMID: 12844146

21. Wang J, Zhou eY, Wu GS. Bim protein degradation contributes to cisplatin resistance. J Biol Chem. 2011; 286: 22384–22392. doi: 10.1074/jbc.M111.239566 PMID: 21561860

22. Ajabnoor GM, Crook T, Coley HM. Paclitaxel resistance is associated with switch from apoptotic to autophagic cell death in MCF-7 breast cancer cells. Cell Death Dis. 2012; 3: e260. doi:10.1038/cddis.2011.139 PMID: 22278287

23. Costa DB, Halmos B, Kumar A, Schumur ST, Huberman MS, Boggon TJ, et al. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med. 2007; 4: 1669–1679. PMID: 17973572

24. Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimic. Proc Natl Acad Sci U S A. 2006; 103: 14907–14912. PMID: 16997913

25. Cragg MS, Kuroda J, Puthalakath H, Huang DC, Strasser A. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. PLoS Med. 2007; 4: 1681–1689. PMID: 17973573

26. Deng J, Shimamura T, Perner S, Carlsson NE, Cai D, Shapiro GI, et al. Proapoptotic BH3-only BCL-2 family protein BIM connects death signaling from epidermal growth factor receptor inhibition to the mitochondrion. Cancer Res. 2007; 67: 11867–11875. PMID: 18069817

27. Gong Y, Somwar R, Politi K, Balak M, Chmielecki J, Jiang X, et al. Induction of BIM is essential for apoptosis triggered by EGFR kinase inhibitors in mutant EGFR-dependent lung adenocarcinomas. PLoS Med. 2007; 4: e294. PMID: 17973573

28. Rahmani M, Anderson A, Habibi JR, Crabtree TR, Mayo M, Harada H, et al. The BH3-only protein Bim plays a critical role in leukemia cell death triggered by concomitant inhibition of the PI3K/Akt and MEK/ERK1/2 pathways. Blood. 2009; 114: 4507–4516. doi: 10.1182/blood-2008-09-177881 PMID: 19773546

29. Wickenden JA, Jin H, Johnson M, Gillings AS, Newson C, Austin M, et al. Colorectal cancer cells with the BRAF(V600E) mutation are addicted to the ERK1/2 pathway for growth factor-independent survival and repression of BIM. Oncogene. 2008; 27: 7150–7161. doi: 10.1038/onc.2008.335 PMID: 18806830

30. Cragg MS, Jansen ES, Cook M, Harris C, Strasser A, Scott CL. Treatment of B-RAF mutant human tumor cells with a MEK inhibitor requires Bim and is enhanced by a BH3 mimic. J Clin Invest. 2008; 118: 3651–3659. doi: 10.1172/JCI35437 PMID: 18949058

31. Lewis JS, Meek KE, Osipo C, Ross EA, Kidawi N, Li T, et al. Intrinsic mechanism of estradiol-induced apoptosis in breast cancer cells resistant to estrogen deprivation. J Natl Cancer Inst. 2005; 97: 1746–1759. PMID: 16333030

32. Tanizaki J, Okamoto I, Fumita S, Okamoto W, Nishio K, Nakagawa K. Roles of BIM induction and survivin downregulation in lapatinib-induced apoptosis in breast cancer cells with HER2 amplification. Oncogene. 2011; 30: 4097–4106. doi: 10.1038/onc.2011.111 PMID: 21499301