The clinicopathological significance of FHIT hypermethylation in non-small cell lung cancer, a meta-analysis and literature review

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Emerging evidence indicates that FHIT is a candidate tumor suppressor in non-small cell lung cancer (NSCLC). However, the correlation between FHIT hypermethylation and clinicopathological characteristics of NSCLC remains unclear. Thus, we conducted a meta-analysis to quantitatively evaluate the effects of FHIT hypermethylation on the incidence of NSCLC and clinicopathological characteristics. Final analysis of 1717 NSCLC patients from 16 eligible studies was performed. FHIT hypermethylation was found to be significantly higher in NSCLC than in normal lung tissue, the pooled OR from 8 studies including 735 NSCLC and 708 normal lung tissue, OR = 5.45, 95% CI = 2.15–13.79, \( p = 0.0003 \). FHIT hypermethylation was also correlated with sex status, smoking status, as well as pathological types. We did not find that FHIT hypermethylation was correlated with the differentiated types or clinical stages in NSCLC patients. However, patients with FHIT hypermethylation had a lower survival rate than those without, HR = 1.73, 95% CI = 1.10–2.71, \( p = 0.02 \). The results of this meta-analysis suggest that FHIT hypermethylation is associated with an increased risk and worsen survival in NSCLC patients. FHIT hypermethylation, which induces the inactivation of FHIT gene, plays an important role in the carcinogenesis and clinical outcome and may serve as a potential drug target of NSCLC.

Lung cancer is the most frequent cause of cancer-related death in many countries, including China1,2. Lung cancers consist of two major histological types, small cell lung carcinoma (SCLC) and non-small cell lung carcinoma (NSCLC); the latter consists of squamous cell carcinoma (SCC), adenocarcinoma (AC), large cell carcinoma and others. NSCLC accounts for approximately 85% of all lung cancers, and there are approximately 80% of NSCLC cases in advanced stage where the prognosis remains poor3. Therefore, investigation of the mechanism of initiation, progression, and identification of prognostic markers is still needed for the selection of patients with NSCLC in order to provide better individualized treatment. Epigenetic modification of gene expression plays an important role in carcinogenesis. Aberrant methylation of CpG dinucleotides is a commonly observed epigenetic modification in human cancer4–6. Thus, the analysis of specific gene promoter methylation as a tool for diagnosis of tumors or its use as prognostic marker has been widely used for many different cancers including NSCLC7.

Fragile histidine triad protein (FHIT), also known as Bis (5’-adenosyl)-triphosphatase, is one of the histidine triad gene family members and is an enzyme encoded by the FHIT gene8,9. Previous reports showed that FHIT was inactivated by the loss of heterozygosity and methylation in cancer cells, which indicated that FHIT is a tumor suppressor protein10,11. Its precise function has been intensively studied in several tumors by the upregulation of inducing cell cycle arrest, apoptosis, inhibition of cell proliferation and by increasing its sensitivity to DNA damaging agents12–14. Lack of protein expression of FHIT by promoter methylation (hypermethylation) has been found to play an important role in lung alveolar differentiation regulation and epithelial tumorigenesis15–18. Although previous studies indicated that inactivation of the FHIT is mainly induced by hypermethylation of FHIT gene, the reported rates of FHIT hypermethylation in NSCLC were remarkably diverse. Moreover, whether it is associated with the incidence and clinical characteristics of NSCLC remains unclear. The variety of the study

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results underpin the need for assessing the evidence of the relationship between FHIT inactivation and NSCLC. Hence, we conducted a systematic review and meta-analysis to quantitatively evaluate the effects of FHIT hypermethylation on the incidence and clinical characteristics of NSCLC.

Results

Identification of relevant studies. Fifty eight publications were identified by the search method as described above. Forty two of those were excluded due to laboratory studies, non-original articles (review), or studies irrelevant to the current analysis. Eventually, there were sixteen studies included in final meta-analysis as shown in Fig. 1. We used Cohen’s kappa statistic to measure the agreement in the most important step for selecting eligible studies between two researchers and showed kappa value 0.76, indicating substantial observer agreement. Of the sixteen studies, ten scored 8 points, six scored 7 points. Hence, the studies were of a relatively high quality (Table 1).

Study characteristics. Sixteen studies published from 2001 to 2011 were eligible for meta-analysis. A total of 1717 NSCLC patients from China, South Korea, Japan, Italy, and USA was enrolled. Their basic characteristics are summarized in Table 2.

The correlation of FHIT hypermethylation with clinicopathological features.

1. The inactivation of FHIT through hypermethylation in NSCLC.

| Study                  | Language | Selection | Comparability | Exposure | Total score |
|------------------------|----------|-----------|---------------|----------|-------------|
| Zöchbauer-Müller, et al. 2001 | English | 3         | 2             | 3        | 8           |
| Hsu, et al. 2007 | English | 3         | 2             | 3        | 8           |
| Yanagawa, et al. 2011 | English | 3         | 2             | 3        | 8           |
| Song, et al. 2011 | Chinese  | 3         | 2             | 2        | 7           |
| Li, et al. 2010 | English | 3         | 2             | 3        | 8           |
| Li, et al. 2009 | Chinese  | 3         | 2             | 2        | 7           |
| Verri, et al. 2009 | English | 3         | 2             | 3        | 8           |
| Yanagawa, et al. 2007 | English | 3         | 2             | 2        | 7           |
| Kim, et al. 2007 | English | 3         | 2             | 2        | 7           |
| Kim, et al. 2006 | English | 3         | 2             | 3        | 8           |
| Nakata, et al. 2005 | English | 3         | 2             | 3        | 8           |
| Ioopoulos, et al. 2005 | English | 3         | 2             | 3        | 8           |
| Tomizawa, et al. 2004 | English | 3         | 1             | 3        | 7           |
| Tzao, et al. 2004 | English | 3         | 2             | 3        | 8           |
| Kim, et al. 2004 | English | 3         | 2             | 2        | 7           |
| Maruyama, et al. 2004 | English | 3         | 2             | 3        | 8           |
| Study                  | Country  | Patients | Methods                      | Primary Aim                                                                 | Methylation site                  | FHIT expression |
|-----------------------|----------|----------|------------------------------|----------------------------------------------------------------------------|-----------------------------------|-----------------|
| Zochbauer-Müller et al. 2001 | United States | 107      | Methylation specific PCR (MSP)/Northern blot analysis | Determine the correlation of protein and hypermethylation status of FHIT in lung and breast cancer | Promoter, CpG islands | +               |
| Hsu et al. 2007       | China    | 63       | MSP                          | Determine the frequency of six genes' hypermethylation in NSCLC              | Promoter, CpG islands | –               |
| Yanagawa et al. 2011  | Japan    | 62       | MSP                          | Determine the methylation status of Multiple genes in NSCLC                 | Promoter, CpG islands | –               |
| Song et al. 2011      | China    | 78       | MSP/RT-PCR                   | Aims to determine the methylation status of five tumor suppressor in NSCLC  | Promoter, CpG islands | +               |
| Li et al. 2010        | China    | 123      | MSP                          | Determine the methylation status of FHIT in NSCLC                          | Promoter, CpG islands | –               |
| Li et al. 2009        | China    | 52       | MSP/RT-PCR                   | Explore the effects of CpG island methylation on protein and mRNA expression of FHIT in NSCLC | Promoter, CpG islands | +               |
| Verri et al. 2009     | Italy    | 187      | MSP/Immunohistochemistry     | Determine the inactivation of FHIT in NSCLC                                | Promoter, CpG islands | +               |
| Yanagawa et al. 2007  | Japan    | 101      | MSP                          | Determine the methylation status of ten genes in pathogenesis of NSCLC     | Promoter, CpG islands | –               |
| Kim et al. 2007       | South Korea | 99        | MSP                          | Determine methylation patterns of eight tumor suppressor gene in NSCLC     | Promoter, CpG islands | –               |
| Kim et al. 2006       | South Korea | 335      | MSP                          | The methylation profile of 5 genes for NSCLC were analyzed and correlated with clinical data | Promoter, CpG islands | –               |
| Nakata et al. 2005    | Japan    | 139      | MSP/Immunohistochemistry     | Determine the inactivation of CDH1, p16 and FHIT in NSCLC                   | Promoter, CpG islands | +               |
| Iliopoulos et al. 2005 | United States | 24        | MSP/Immunohistochemistry     | Determine the inactivation of FHIT and WWOX in lung, breast and bladder cancer | Promoter, CpG islands | +               |
| Tomizawa et al. 2004  | Japan    | 54       | MSP                          | Investigate the clinicopathological significance of aberrant methylation of RARβ/2, RASSF1A and FHIT in NSCLC patients | Promoter, CpG islands | –               |
| Tzao et al. 2004      | China    | 44       | MSP/RT-PCR                   | Examines protein, mRNA expression, and hypermethylation of the FHIT gene in NSCLC | Promoter, CpG islands | +               |
| Kim et al. 2004       | South Korea | 125      | MSP                          | Determine the clinicopathological and prognostic significance of FHIT methylation in NSCLC | Promoter, CpG islands | –               |
| Maruyama et al. 2004  | United States | 124      | MSP                          | Determine the correlation between the aberrant promoter methylation of multiple genes and survival in patients with NSCLC | Promoter, CpG islands | –               |

Table 2. Basic characteristics of the included studies.

We first determined that FHIT hypermethylation was significantly higher in NSCLC than in normal lung tissues. The pooled OR from 8 studies including 735 NSCLC and 708 normal lung tissues, is shown in Fig. 2A (OR = 5.45, 95% CI = 2.15-13.79, p = 0.0003), indicating that FHIT inactivation through hypermethylation plays an important role in the carcinogenesis of NSCLC. Since the heterogeneity is very high (I² = 84%), we deleted one study (Verri 2009), recalculated the pooled OR from remaining 7 studies and shown in Fig. 2B. I² dramatically reduced to 14%, indicating that the heterogeneity is very low.

2. Relationship between the frequency of FHIT hypermethylation and sex status.

Next, we determined whether FHIT hypermethylation rate was correlated with sex status. The pooled OR from 7 studies including 722 males and 290 females’ NSCLC, as shown in Fig. 3 (OR = 1.38, 95% CI = 1.02-1.87, p = 0.04), that indicate that FHIT hypermethylation was correlated with sex status in which it is higher in male than in female.

3. Relationship between the frequency of FHIT hypermethylation and smoking status.

Then, we determined whether FHIT hypermethylation rate was correlated with smoking status. The pooled OR from 9 studies including 268 and 809 NSCLC with and without smoking history is shown in Fig. 4 (OR = 0.69, 95% CI = 0.51-0.93, p = 0.02), indicates that FHIT hypermethylation is correlated with smoking status in NSCLC patients.

4. Relationship between the frequency of FHIT hypermethylation and pathological types.

We also determined whether FHIT hypermethylation was correlated with pathological types. The pooled OR from 8 studies including 490 squamous cell carcinoma (SCC) and 494 adenocarcinoma (AD), is shown in Fig. 5 (OR = 1.49, 95% CI = 1.14-1.95, p = 0.004), which indicates that FHIT hypermethylation plays a more important role in the pathogenesis of SCC.

5. The role of FHIT hypermethylation in NSCLC progression.

We analyzed 366 NSCLC patients pooled from 3 studies to assess whether the aberrant FHIT hypermethylation in NSCLC was associated with the differentiated status. As shown in Fig. 6A, aberrant FHIT hypermethylation is not significantly higher in poorly differentiated NSCLC than that in moderately or highly differentiated NSCLC, OR = 1.30, 95% CI = 0.80-2.09, p = 0.29. In addition, aberrant FHIT hypermethylation...
hypermethylation is also not significantly higher in advanced NSCLC (III & IV) than that in early staged NSCLC (I & II), OR = 1.04, 95% CI = 0.77–1.41, p = 0.79, Fig. 6B. These results suggest that FHIT hypermethylation may not play an important role in NSCLC progression and different stages.

6. FHIT hypermethylation as a prognostic factor for NSCLC.

There are 4 studies estimating the relationship between FHIT hypermethylation and overall survival (OS) in NSCLC patients. The pooled HR for OS shows that FHIT hypermethylation is associated with worsen survival in NSCLC patients as shown in Fig. 7 (HR = 1.73, 95% CI = 1.10–2.71, p = 0.02).

7. Sensitivity analyses and publication bias.

A sensitivity analysis, in which one study was removed at a time, was conducted to assess the result stability. In the case of relationship between FHIT hypermethylation in NSCLC and in normal lung tissue, the overall OR are in the range from 3.82 (95% CI: 1.31–11.15 to 110.03 (95% CI: 6.167–1814.87). The pooled ORs and HRs are not significantly changed, indicating the stability of our analyses. The funnel plots are largely symmetric, (Fig. 8A–G).

Figure 2. The pooled OR from 8 studies included 735 NSCLC and 708 normal lung tissues, I² = 84%; OR = 5.45, 95% CI = 2.15–13.79, p = 0.0003. (A) The pooled OR from 7 studies included 548 NSCLC and 500 normal lung tissues, I² = 14%; OR = 8.08, 95% CI = 5.24–12.47, p < 0.00001 (B).

Figure 3. The pooled OR from 7 studies included 722 males and 290 females’ NSCLC, OR = 1.38, 95% CI = 1.02–1.87, p = 0.04, which indicates that FHIT hypermethylation is correlated with sex status in NSCLC patients.
suggesting there are no publication biases in the meta-analysis of FHIT hypermethylation and clinicopathological features.

**Discussion**

Systematic reviews and meta-analyses have become increasingly important in biomedical science. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (the PRISMA) are recommended to authorize the readers to access the strengths and weaknesses of the study.

**Interpretation of results and comparison with other studies.** The FHIT gene locates the most common fragile site in the human genome, FRA3B (3p14.2), in which undergoes genomic rearrangement, biallelic loss, and cytogenetic abnormalities in tumors8,34,35. FHIT is genetically or epigenetically altered in many primary and advanced carcinomas. Inactivation of FHIT by promoter hypermethylation plays an important role in tumorigenesis in several types of tumors including NSCLC27,36–44. To date, there have been some studies describing the methylation status of FHIT in NSCLC; however, the roles of methylation of FHIT in NSCLC and clinical significance have not been thoroughly investigated. We conducted the meta-analysis to determine the correlation between FHIT hypermethylation and clinicopathological characteristics in NSCLC. Analysis of the pooled data showed that (1) NSCLC has a higher hypermethylation than normal lung tissue; (2) FHIT hypermethylation is correlated with sex status in which it is higher in male than in female. (3) FHIT hypermethylation is correlated with smoking status in NSCLC patients. (4) FHIT hypermethylation is correlated with pathological types and plays a more important role in the pathogenesis of SCC. (5) FHIT hypermethylation is not significantly higher in

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**Figure 4.** 1077 NSCLC patients with the smoking status pooled in 9 studies. Aberrant FHIT hypermethylation is correlated with the smoking status in NSCLC patients, $=0.69$, 95% CI $=0.51–0.93$, $p=0.02$.

**Figure 5.** The pooled OR from 8 studies included 490 squamous cell carcinoma (SCC) and 494 adenocarcinoma (AD), OR $=1.49$, 95% CI $=1.14–1.95$, $p=0.004$, indicates that FHIT hypermethylation plays more important role in the pathogenesis of SCC.
poorly differentiated NSCLC than that in moderately or highly differentiated NSCLC. In addition, \( \text{FHIT} \) hypermethylation is also not significantly higher in advanced NSCLC (III & IV) than that in early staged NSCLC (I & II). (6) The pooled HR for OS shows that \( \text{FHIT} \) hypermethylation is associated with worse survival in NSCLC patients. The cumulative evidence in our study is now conclusive that the \( \text{FHIT} \) gene promoter hypermethylation is associated with lung cancer formation and development, male gender, smoking behavior, and worse survival. In a meta-analyses of the gene methylation versus the cigarette smoking in NSCLC patients by Huang et al. \(^{45}\), \( \text{FHIT} \) methylation was found to be significantly associated with the smoking behavior, which support our conclusions. However, unavailability of meta-analysis or systemic review on other particular outcomes such as NSCLC initiation and development, gender and survival status makes it impossible to compare our results with other similar studies. The results suggest a potential role of \( \text{FHIT} \) methylation analysis in diagnosis and prognosis of lung cancer in clinical settings. Epigenetic alteration, particularly aberrant DNA methylation, is one of the best-characterized epigenetic modifications that contribute to tumor initiation and progression.\(^5,6\). FHIT
is thought to affect cellular function and behavior largely through its signaling properties. FHIT also activates caspase-8 and caspase-2, which causes the release of cytochrome c and finally induces apoptosis\(^46\). FHIT and p53, the two most commonly altered tumor suppressor genes, might rely on common mediators and crosstalk among these proteins in regulation of growth-related pathways; thus, the inactivation of both genes results in prominent deregulation of cell proliferation and tumor progression in lung cancer\(^47\). Huang et al. showed that 7 hypermethylated genes including FHIT were significantly associated with the smoking behavior in NSCLC patients\(^45\). The difference about the result may be due to the different selected number of studies. They selected only 5 studies which included 518 patients. Our studies searched 9 studies which included 1077 patients. A number of studies showed that inactivation of FHIT can cause tumor aberrant progression and link to clinicopathological characteristics\(^27,48–51\). Therefore, FHIT can be considered as a tumor suppressor, and its inactivation could contribute tumor progression and poor prognosis. Although only four studies evaluated the relationship between overall survival and FHIT hypermethylation in NSCLC, they showed very similar results\(^25,27,28,32\). Based on this meta-analysis, the pooled HR for OS showed that FHIT hypermethylation was associated with worsen survival in NSCLC patients, \(HR = 1.73, 95\% CI = 1.10–2.71, p = 0.02\). Therefore, we may consider that FHIT hypermethylation in NSCLC tends to indicate a poor prognosis.

**Strengths and limitations of evidence.** In the comparison cancer and normal lung tissue, the heterogeneity is very high (\(I^2 = 84\%\)), thus we deleted one study (Verri 2009)\(^16\), re-calculated the pooled OR from remaining 7 studies and shown in Fig. 2B. \(I^2\) dramatically reduced to 14%, indicating that the heterogeneity is very low. The reason of their results were total different from other studies is not clear, they could have used inappropriate primers and methylation specific PCR (MSP) condition in detection of FHIT hypermethylation. Consistent results were shown in sensitivity analyses, and no evidence of publication bias was found. This study has several potential limitations. First, the possibility of information and selection biases and unidentified confounders could not be completely excluded because all of the included studies were observational. Second, the searching strategy was restricted to articles published in English. Articles with potentially high-quality data

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**Figure 8.** The funnel plots are largely symmetric, which suggests that there are no publication biases in the meta-analysis of FHIT hypermethylation and clinicopathological features. The funnel plot from 8 studies comparing NSCLC and normal lung tissue (A). The funnel plot from 7 studies determined the relationship between FHIT hypermethylation and the sex status in NSCLC patients (B). The funnel plot from 9 studies determined the relationship between FHIT hypermethylation and the smoking status in NSCLC patients (C). The funnel plot from 8 studies comparing FHIT hypermethylation between squamous cell carcinoma (SCC) and adnocarcinoma (AD) (D). The funnel plot from 3 studies determined FHIT hypermethylation in different differentiated NSCLC (E). The funnel plot from 7 studies determined FHIT hypermethylation in different staged NSCLC (F). The funnel plot from 4 studies determined the relationship between FHIT hypermethylation and overall survival (OS) in NSCLC (G).
that were published in other languages were not included because of the anticipated difficulties in obtaining accurate medical translation. Hence, cautions should be taken when our findings are interpreted among the general populations.

**Research and Clinical implications.** The results from the current study demonstrate that the hypermethylation rate of FHIT gene promoter in NSCLC is significantly higher than that in the normal lung tissues, indicating that FHIT promoter hypermethylation is common in NSCLC. Since changes in FHIT promoter hypermethylation are reversible, drug treatment through demethylation may be useful to delay carcinogenesis and progression and to improve prognosis. Lung cancer cell clones carrying conditional FHIT transgenes showed significant suppression of xenograft tumor growth, suggesting that treatments to restore endogenous FHIT expression in lung cancers would result in decreased tumorigenicity \(^{17}\). In addition, injection of 5-aza-2-deoxycytidine (AZA) and trichostatin A (TSA) in nude mice with established H1299 tumors showed suppressed growth of small tumors without apparent toxicity, and responding tumors showed restoration of FHIT\(^{17}\). These preclinical studies show the therapeutic potential of restoration of tumor suppressor expression through epigenetic modulation. This approach may bring new direction and hope for cancer treatment through gene-targeted therapy.

In conclusion, our meta-analysis shows that NSCLC had a higher FHIT hypermethylation than normal lung tissue, higher in male than in female, higher in non-smoker than in smoker, and higher in SCC than in AD. In addition, FHIT hypermethylation is associated with an increased risk and worsen survival in NSCLC. Further large-scale studies, especially multi-center and well-matched cohort research, will provide more insight into the role of FHIT in the prognosis and clinical implementation of NSCLC patients.

**Material and Methods**

**Information sources.** Key database searched, data extraction and methodological assessment. We searched Pubmed, Embase, and ISI web of knowledge to identify studies from May 1, 1998 to March 1, 2014 using the search terms: “lung” and “cancer or tumor or neoplasm or carcinoma”, “methylation”, and “FHIT or Fragile histidine triad protein or Bis (5’-adenosyl)-triphosphatase”. We also searched manually for the reference lists of the retrieved articles and reviews for additional articles.

Although our search did not have language limits initially, for the full-text reading and final evaluation we only performed the review of the studies published in English language. After excluding non-relevant and/or redundant publications from different databases, the remaining papers were evaluated in the full text version for in- and exclusion criteria and for relevant articles in the reference lists. All searched data were retrieved. Authors' bibliographies and references of selected studies were also searched for other relevant studies. The most complete study was chosen to avoid duplication if same patient populations were reported in several publications.

Two authors (WY, NX) independently reviewed and extracted data from eligible studies. Disagreements were resolved by discussion and consensus with a third author (BH). The following information was recorded for each study: the first author name, year of publication, sample source, number of cases, clinicopathological parameters, cancer TNM (tumor node metastasis) stage, methylation detection method, methylation rate and/or expression, and follow up. Data for study characteristics and clinical responses were summarized and organized into a table format. Heterogeneity of investigation was evaluated to determine whether the data of the various studies could be analyzed for a meta-analysis.

For the methodological evaluation of the studies, three investigators (XZ, XH and BH) read through each publication independently, and they assessed and scored studies according to the Newcastle-Ottawa Scale (NOS)\(^{35}\), which was developed to assess the quality of nonrandomised studies with its design, content and ease of use directed to the task of incorporating the quality assessments in the interpretation of our meta-analytic results (Table 1). The three readers provided the quality scores and compared them, and then they reached a consensus value for each item.

**Eligibility criteria.** Criteria that an eligible study has to meet were as follows: (1) FHIT hypermethylation evaluated in the primary NSCLC tissues, (2) researches revealed the relationship between FHIT hypermethylation and NSCLC clinicopathological parameters and prognosis, (3) FHIT hypermethylation examined by polymerase chain reaction (PCR), (4) studies provided sufficient information to estimate hazard ratio (HR) about overall survival (OS) and 95% confidence interval (CI). The exclusion criteria included the following: (1) letters, reviews, case reports, conference abstracts, editorials, expert opinion, (2) all publications regarding overall survival (OS) and 95% confidence interval (95% CI) were estimated. The frequency of FHIT hypermethylation was compared in different tumor characteristics. Heterogeneity among studies was evaluated with Cochran's Q test\(^{36}\) and the \(I^2\) statistic\(^{36,57}\). When heterogeneity was not an issue (\(P\) values < 0.05), a fixed effect model was used to calculate parameters. If there was substantial heterogeneity (\(I^2\) values \(\geq 50\%\)), a random-effects model was used to pool
data and attempt to identify potential sources of heterogeneity based on subgroup analyses. The pooled OR was estimated for the association between FHIT hypermethylation and clinicopathological features. P values tailored less than 0.05 were considered statistically significant.

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**Author Contributions**

W.Y. and B.H. participated in the design of the study and identify related studies, as well as drafted the manuscript. W.Y., N.X., X.H. and X.Z. reviewed and extracted data from eligible studies. W.Y., X.H. and B.H. participated in the search the study and performed the statistical analysis.

**Additional Information**

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