**Meta-analysis of Associations of the Ezrin Gene with Human Osteosarcoma Response to Chemotherapy and Prognosis**

Zhe Wang, Mao-Lin He, Jin-Min Zhao, Hai-Hui Qing, Yang Wu

**Abstract**

Various studies examining the relationship between Ezrin overexpression and response to chemotherapy and clinical outcome in patients with osteosarcoma have yielded inconclusive results. We accordingly conducted a meta-analysis of 7 studies (n = 318 patients) that evaluated the correlation between Ezrin and histologic response to chemotherapy and clinical prognosis (death). Data were synthesized in receiver operating characteristic curves and with fixed-effects and random-effects likelihood ratios and risk ratios. Quantitative synthesis showed that Ezrin is not a prognostic factor for the response to chemotherapy. The positive likelihood ratio was 0.538 (95% confidence interval [95% CI], 0.296-0.979; random-effects calculation), and the negative likelihood ratio was 2.151 (95% CI, 0.905-5.114; random-effects calculations). There was some between-study heterogeneity, but no study showed strong discriminating ability. Conversely, Ezrin positive status tended to be associated with a lower 2-year survival (risk ratio, 2.45; 95% CI, 1.26-4.76; random-effects calculation) with some between-study heterogeneity that disappeared when only studies that employed immunohistochemistry were considered (risk ratio, 2.97; 95% CI 2.01-4.40; fixed-effects calculation). To conclude, Ezrin is not associated with the histologic response to chemotherapy in patients with osteosarcoma, whereas Ezrin positivity was associated with a lower 2-year survival rate regardless of risk of death at 2 years. Expression change of Ezrin is an independent prognostic factor in patients with osteosarcoma.

**Keywords:** Ezrin - osteosarcoma - chemotherapy - survival - meta-analysis

**Introduction**

Osteosarcoma is a life-threatening malignancy that most often occurs in teenagers (Ottaviani and Jaffe, 2009). The prognosis of patients with osteosarcoma remains poor, in spite of advances in surgical treatment and the availability of new chemotherapeutic agents in recent decades. The overall relapse-free survival rate over 5 years is approximately 65% (Foster et al., 2007; Gorlick, 2009). Ezrin also known as Villin2 (Vil2), located in the 6q25-6q26 of human chromosome, about 24kb and contains 13 exons. Its mRNA full length is 3166 bp. Located in between D6S442 and D6S281 (Hunter, 2004). Ezrin’s relative molecular weight is about 81KD and composed of 585 amino acids, so it’s the members of the ERM (Ezrin – Radixin – Moesin) family (Bretscher et al., 2002). ERM family is the member of band4.1 family, so its homology up to 75 ~ 80% with other members, with similar structure, as the connection of cytomembrane and actin cytoskeleton (Hunter, 2004). Several studies have tried to investigate the clinical significance of Ezrin overexpression in osteosarcoma. Recent studies have come to inconsistent conclusions. In some studies, the increased levels of Ezrin protein at diagnosis resulted in an association with a worse clinical outcome (Kim et al., 2007; Xu-dong et al., 2008; Kim et al., 2009; Xu-Dong et al., 2009), whereas other reports either did not show any correlation between Ezrin and prognosis (Salas et al., 2007, Boldrini et al., 2010) or indicated a positive prognostic value for increased Ezrin expression at diagnosis (Wang et al., 2011). Most studies had a limited sample size, thus a quantitative synthesis using rigorous methods would be important to perform. We accordingly conducted a meta-analysis of all available studies relating Ezrin expression and Ezrin gene alterations with response to chemotherapy and/or clinical outcome, as defined by 2-year survival, because all studies were longitudinal over at least a 2-year period.

**Materials and Methods**

Identification and eligibility of relevant studies

We searched electronic databases PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and Embase (http://www.embase.com/) updated on Dec 2012 for all publications on the association of Ezrin expression with osteosarcoma outcomes. The search strategy was based on
combinations of the terms “osteosarcoma”, “osteogenic sarcoma”, “ezrin”. Investigators were contacted and asked to supply additional data when key information relevant to the meta-analysis was missing.

No language or country restrictions were applied. All eligible studies were retrieved, and their bibliographies were checked for other relevant publications. Review articles and bibliographies of other relevant studies identified were searched by hand to find additional eligible studies. The inclusion criteria were as follows: (a) studies examining the relation of Ezrin status to response to chemotherapy and/or clinical outcome (death), (b) studies measuring Ezrin status with the method of immunohistochemistry (IHC) for protein levels or reverse transcription-PCR (RT-PCR) techniques for identifying gene changes, (c) cases were medically confirmed of osteosarcoma, (d) reported outcome measures with Kaplan–Meier curves or 2-year survival rate, (e) case – control and cohort studies.

Whenever studies pertained to overlapping patients, we retained only the largest study to avoid duplication of information.

Definition and standardizations

For consistency, we use “Ezrin” for the gene name, “Ezrin” for the expressed protein, and “Ezrin status” for covering both the gene and protein markers. All studies to be considered by the overall analysis, regardless of whether protein expression or mutants were being evaluated, but we also performed separate analyses for Ezrin protein expression and Ezrin gene alterations. In the overall analysis, for studies using both IHC and RT-PCR, we used the IHC data. For studies using IHC, we used prespecified rules to standardize, as much as possible, the definition of a positive test for studies that used different cutoff thresholds. We defined Ezrin protein positivity as nuclear cell stain in at least more than 0% of the tumor cells, a definition followed by most studies. When different definitions were used, we accepted the cutoff closest to the 10% level.

We defined “response to chemotherapy” by the percentage of histologic necrosis of tumor cells in specimens obtained after chemotherapy. A cutoff of 90% necrosis was used to separate responders from nonresponders.

The clinical outcome of interest was mortality. Clinical outcomes were standardised to include a 24 month follow-up across all studies to avoid large time differentiation between studies. All studies had at least 24 months of follow-up.

Data extraction

Two investigators extracted data from eligible studies independently, discussed discrepancies and reached consensus for all items. We extracted data on characteristics of studies and patients, measurements, and results. For each report, we recorded author’s names, journal and year of publication, country of origin, years of patient enrollment, number of patients analyzed, stage and grade of osteosarcoma, demographics, chemotherapy and surgery used, timing of Ezrin assessment (prechemotherapy or postchemotherapy), type of Ezrin measurements, antibodies used for IHC, and definition(s) of Ezrin positivity. Data on the main outcomes were entered in 2x2 tables showing the histologic response/nonresponse to chemotherapy and whether death occurred within 24 months per Ezrin status.

Quality assessment

We assessed the methodological quality of included studies based on Newcastle–Ottawa scale (NOS) for quality of case – control and cohort studies (Stang, 2010). A star system of the NOS (range, 0–9 stars) has been developed for the evaluation. The highest value for quality assessment was 9 stars (Table 1).

Statistical analysis

The Summary Receiver Operating Characteristic (SROC) curve and the combined positive and negative likelihood ratios (LR + and LR -, respectively) were applied to evaluate data on the diagnostic performance of Ezrin for determining histologic response to chemotherapy.

For a diagnostic or predictive test, the sensitivity (true positives) and specificity (1-false positive) are correlated; therefore, it is not correct to estimate these two quantities independently. To bypass this problem, the SROC method was used. The SROC curve is estimated by the regression D=A+B*S, where D is the difference of the logits of the true-positive and false-positive rate, and S is the sum of these logits (Moses et al., 1993). The SROC curve shows the balance between sensitivity and specificity across the included studies.

Likelihood ratios, also metrics, that combine both sensitivity and specificity in their calculation. LR + is defined as the ratio of sensitivity over 1 - specificity, whereas LR - is defined as the ratio of 1 - sensitivity over specificity. When there is absolutely no discriminating ability for a diagnostic or predictive test, both LRs = 1. The higher the LR + and the lower the LR -, the better the discriminating ability. Although there is no absolute cutoff level, a good diagnostic test may have LR + > 5 and LR - < 0.2. Study specific LR values were combined with fixed-effects and random-effects models, and between-study heterogeneity was assessed with the Q statistic (Petitti, 2000).

Data on the predictive ability of Ezrin for 24-month clinical outcomes were combined across studies in a similar fashion using random-effects estimates for the synthesis of risk ratios for disease progression (Petitti, 2000). The risk ratio shows the rate of 2-year mortality in the group with Ezrin overexpression or Ezrin gene alterations divided by the rate of 2-year mortality in the group without Ezrin expression or Ezrin gene alterations. Between-study heterogeneity in the risk ratios was assessed with the Q statistic (Petitti, 2000).

Fixed-effects models presume that differences between the results of the combined studies are due entirely to chance. Random-effects models allow for the possibility that results may differ genuinely between studies. In the presence of between-study heterogeneity, random-effects models provide wider confidence intervals (CIs) (Lau et al., 1997). We generally present random-effects estimates,
Table 1. Characteristics of Eligible Studies

<table>
<thead>
<tr>
<th>Author (y)</th>
<th>Total score</th>
<th>No. Analyzed</th>
<th>Metastatic disease</th>
<th>Age (y) mean</th>
<th>Ezrin status method</th>
<th>IHC Antibodies</th>
<th>PCR Exons</th>
<th>IHC Cutoff%</th>
<th>Response (criteria)</th>
<th>Deaths In 2 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chan Kim (2009)</td>
<td>8</td>
<td>70</td>
<td>30</td>
<td>16</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erica Boldrini (2010)</td>
<td>9</td>
<td>52</td>
<td>24</td>
<td>16</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min Suk Kim (2007)</td>
<td>6</td>
<td>56</td>
<td>27</td>
<td>22</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shen Xu dong (2008)</td>
<td>7</td>
<td>32</td>
<td>3</td>
<td>18</td>
<td>IHC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sébastien Salas (2007)</td>
<td>8</td>
<td>37</td>
<td>13</td>
<td>15</td>
<td>RT–PCR+IHC</td>
<td>clone 3C12,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Antibodies, antibodies used for detection of Ezrin protein with IHC; Exons, exons of the Ezrin gene analyzed by polymerase chain reaction; Huvos, histological response based on the Huvos grading system; NR, not reported; N90, histological response based on >90% tumor cell necrosis; N95, histological response based on >95% tumor cell necrosis; PCR, polymerase chain reaction

Figure 1. The Process Flow Diagram Describes How We Filtered the Data We Retrieved

unless stated otherwise.

Sensitivity analysis examined the effect of limiting the evaluations studies using the 10% IHC cutoff. If the articles be excluded, the results did not change much, indicating that the sensitivity is low and the result is more robust and credible. On the contrary, if the articles were excluded, the results did change much, indicating that the sensitivity is high and the result is fewer robust and credible.

Funnel plots were created for assessment of possible publication biases. Analyses were conducted in using SPSS (version16.0), Review Manager (version5.0) and Meta-Disc (version1.4) software packages.

Results

Eligible studies

We initially identified 23 reports including the role of Ezrin status in patients with osteosarcoma. Of those, 16 reports were excluded: six due to reviews, nine due to lack of any informative clinical data, and one due to it is overlapping with another study (Figure 1). In all, seven independent eligible studies (Kim et al., 2007; Salas et al., 2007; Xu-dong et al., 2008; Kim et al., 2009; Xu-Dong et al., 2009; Boldrini et al., 2010; Wang et al., 2011), which had data on 2-year survival and enrolled a total of 318 patients, were included in the quantitative synthesis. The mean or median age of patients included in each study ranged from 15 to 22 years across the eligible studies; these populations were young. All analysed new osteosarcoma was treated with combination chemotherapy regimens. Surgery comprised resection, limb salvage, disarticulation, curettage or amputation procedures.

Five studies (Kim et al., 2007; Xu-dong et al., 2008; Salas et al., 2007; Xu-dong et al., 2008; Kim et al., 2009; Xu-Dong et al., 2009) used the cutoff of 95% necrosis and one studies (Boldrini et al., 2010) used more than 1% cut-off Ezrin positivity and two studies (Salas et al., 2007; Boldrini et al., 2010) used more than 10% cut-off Ezrin positivity, whereas two studies (Salas et al., 2007; Boldrini et al., 2010) used more than 1% cut-off Ezrin positivity and two studies (Kim et al., 2007; Xu-dong et al., 2008) used more than 0% cut-off Ezrin positivity. The antibodies to be used in each study are not the same (Table 1). The cutoff of 90% necrosis to separate responders from nonresponders was used by three studies (Kim et al., 2007; Kim et al., 2009; Wang et al., 2011), whereas one study (Salas et al., 2007) used the cutoff of 95% necrosis and one studies (Boldrini et al., 2010) used the Huvos grading system. The incidence of histologic response to chemotherapy ranged from 36% to 53.1%, and 2-year mortality rates ranged between 20% and 61.8% across the eligible studies. Both the chemotherapy response rates and 2-year mortality differed significantly across studies (P < 0.01 for both). This may be due to differences in the case mix of the study populations (e.g., grade and stage) and/or the therapies used.

Data Synthesis: Response to Chemotherapy

Ezrin status had no discriminating ability to identify
poor versus good responders to chemotherapy. When all studies were considered, the SROC curve showed the changing proportion of sensitivity to specificity, suggestive of a total lack of discriminating performance (Figure 2). According to the SROC, a sensitivity of 50% corresponded to a specificity of only 18%, and a specificity of 50% corresponded to a sensitivity of only 18% in the analysis. No study showed any particularly strong discriminating performance overall.

Separate analyses with studies using only IHC were similar (Table 2). In the overall analysis and sensitivity analyses, LR+ remained in the range of 0.538–0.569, and LR- remained in the range of 1.878–2.151, values characteristic of very poor discriminating performance (Table 2). It is noteworthy that there was significance between-study heterogeneity for both metrics, both in the overall analysis and after excluding various studies in sensitivity analyses. There was also no evidence that large studies yielded stronger association of Ezrin positive status with 2-year mortality when compared with smaller studies (\( P < 0.10 \)).

Data synthesis: survival at 2 years

Ezrin positivity was associated with a lower 2-year survival rate regarding the risk of death at 2 years (Figure 3). However, there was significance between-study heterogeneity in these results, the risk of death at 2 years doubled. A sensitivity analysis showed a persistent, increased risk of death at 2 years (Table 3), and between-study heterogeneity was no longer significant when the analyses were limited to IHC studies. Larger studies tended to show stronger association of Ezrin positive status with 2-year mortality when compared with smaller studies (\( P < 0.10 \)).

Publication bias

The funnel plot, to some extent, was symmetric (Figure 4). These results indicated a possibility that publication bias may have played a role in the observed effect, but since negative results were included in the univariate analysis, it was unlikely to make a great sense.

Discussion

To our knowledge, this is the first meta-analysis investigating the association of Ezrin gene with response to chemotherapy and prognosis in human osteosarcoma. This is a very heterogenous disease entity and there are multiple factors that have an influence on the prognosis of osteosarcoma. The most widely used prognostic factor is the histologic response to preoperative chemotherapy. Some patients show a good response to chemotherapy and are eventually cured by subsequent surgery, whereas others show resistance to chemotherapy and the disease rapidly progresses. However, the molecular biomarkers for osteosarcoma are not well known and we continue to carry out much research in the field. It has been studied for Ezrin gene as a prognostic marker in patients with osteosarcoma, reached inconsistent conclusions. This meta-analysis by the statistical study of the response to chemotherapy and 2-year survival, and draw the following two basic conclusions: (1) Ezrin status had no discriminating ability to identify poor versus good responders to chemotherapy. (2) Ezrin positivity was associated with a lower 2-year survival rate.

Ezrin expression has relatively specific of tissues

Table 2. Likelihood Ratios for the Association Between Ezrin Status and no Histologic Response to Chemotherapy

<table>
<thead>
<tr>
<th>Studies</th>
<th>N (n)</th>
<th>Positive LR (95% CI)</th>
<th>Q</th>
<th>Negative LR (95% CI)</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>4(196)</td>
<td>0.538(0.296–0.979)</td>
<td>10.57</td>
<td>2.151(0.905–5.114)</td>
<td>12.81</td>
</tr>
<tr>
<td>IHC only</td>
<td>3(171)</td>
<td>0.569(0.276–1.174)</td>
<td>9.77</td>
<td>1.878(0.669–5.267)</td>
<td>10.54</td>
</tr>
</tbody>
</table>

Sensitivity analysis

All \( P \)-values were \( < 0.01 \) for between-study heterogeneity; All figures are based on random effects calculations; CI, confidence interval; *Only one study with 4 subjects used the 10% cutoff; thus, this sensitivity analysis would not be meaningful.

Table 3. Risk Ratio for Association Between Ezrin Status and Mortality Within 24 Months

<table>
<thead>
<tr>
<th>Studies</th>
<th>N (n)</th>
<th>Risk ratio (95% CI)</th>
<th>Q</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All 7(318)</td>
<td>14.75</td>
<td>2.45 (1.26, 4.76)</td>
<td>14.75</td>
<td>2.45 (1.26, 4.76)</td>
</tr>
<tr>
<td>IHC only 6(293)</td>
<td>8.22</td>
<td>2.97 (2.01, 4.40)</td>
<td>8.22</td>
<td>2.97 (2.01, 4.40)</td>
</tr>
</tbody>
</table>

Specific 10% cutoff 2(102) 2.72 2.33 (1.02, 5.29)

\( 0.01 < P \leq 0.10 \) for between-study heterogeneity. Risk ratio was estimated with random effects models; \( ^a \) \( P > 0.1 \) for between-study heterogeneity; Risk ratio was estimated with fixed effects models; CI, confidence interval

Figure 3. Meta-analysis of the Association Between Ezrin Status and the Risk of Death at 2 Years. Each study is shown by the name of the lead author, year of publication, and the relative risk with 95% CI. Also shown are the summary relative risk (total) and 95% CI with random effect calculations. CI, confidence intervals; df, degree of freedom; RR, risk ratio

Figure 4. Funnel Plot of the Association Between Ezrin Status and the Risk of Death at 2 Years
and cells. It’s distributed in the brain, kidney, intestine, lung, peripheral nerve and Schwann cells of adult tissues. Subcellular located at the cytoplasmic membrane, the apical of the microvilli, actin-containing surface material, and the bond in between of the cell (Sun et al., 2002).

Ezrin is the bridge that connected to the cell membrane and cytoskeleton. It mainly appears in the cell surface. It expresses different function through different molecules signaling of membrane surface and transduction pathways of transmembrane signal (Krishnan et al., 2006). It involved in cell survival, adhesion, proliferation, migration and other process.

Ezrin is known to be a component of cell-surface structures that are involved in cell adhesion to the extracellular matrix, as well as in cell–cell interactions, receptor tyrosine-kinase signaling, signal transduction through Rho GTPase and interactions with the Akt-mediated cellular apoptotic machinery (Gautreau et al., 1999; Martin et al., 2003).

The occurrence, development, invasion and metastasis of malignant tumor are a process that affected by multiple factors. The variety biological function of Ezrin is related to biological characteristics of malignant tumor closely. An increasing number of researches are revealing the relationship between them.

In the recent years, there has been mounting evidence that Ezrin expression enhancement than normal tissue in bladder cancer (Kumar et al., 2003), colorectal cancer (Wang et al., 2009), gastric carcinoma (Shi et al., 2006), breast cancer (Bal et al., 2007), salivary gland adenoid cystic carcinoma (You-yuan et al., 2009) and other cancer. Following studies correlating the expression levels of ezrin to the metastatic potential of different types of tumors, experimental models have demonstrated the implication of ezrin in the metastatic spread of osteosarcoma, rhabdomyosarcoma and mammary tumor cells (Khanna et al., 2004; Yu et al., 2004; Elliott et al., 2005).

Ezrin’s intracellular localization is varies due to the benign cells or the malignant cells. Generally, Ezrin is mainly distributed in the cell membrane. But in malignant cells, it’s scattered in the cytoplasm. Ezrin’s function can be express through the combination of E-cadherin (Vaheri et al., 1997, Hong-Jian et al., 2007, Wang et al., 2009), Lamp-1 (Brambilla and Fais, 2009; Federici et al., 2009), merlin (McClatchey, 2003; Federici et al., 2009) molecules or co-expression. And turn polar distribution in normal cell membrane become distributed in the malignant tumor. Meanwhile, it can weaken the adhesion between the normal cells, enhancing adhesion between tumor and other cells which promoting the invasion and metastasis of the tumor. Ezrin also enhanced cell viability and viability, decreased cell death, to avoid anoikis through MAPK, Akt and other approach. It’s conducive to tumor cell survival in the adverse environment, and enhanced malignant tumor invasiveness. Studies consider that the participated of Ezrin is needed in tumor metastasis. Although it can’t be prove to be the dominant factor of transfer.

Ezrin positivity was associated with a lower 2-year survival rate, this may be due to the overexpression of Ezrin protein leads to tumor metastasis ability enhancement. But the mechanism is still not very clear.

Ezrin protein can act directly a part in the hyaluronic acid receptor CD44 in the cytoplasmic portion, while CD44 molecules overexpression can cause Ezrin functional activation, thereby promoting tumor cell metastasis ability enhancement (Martin et al., 2003). In addition, Ezrin protein can also be through synergy and amplifying the cell surface of tumor metastasis-related signal, changing balance of intracellular signal transduction, involved in tumor metastasis mechanism. Ezrin can be used as tyrosine-phosphate kinase substrates in transmembrane signal, playing a role in the regulation of signal transduction and cell response to the signal. Khanna at al. (2004) research found that MAPK to promote cell survival and Akt activity is reduced with the decreased expression of Ezrin. Late metastasis of the tumor process, not Akt but the MAPK pathway increased the transfer advantage of Ezrin low-expressing cells, indicating that Ezrin may regulate several signaling pathways to promote tumor metastasis.

Recently, many meta-analyses have been performed to investigate the association between many genes (VEGF (Qu et al., 2012), HER-2 (Li and Geng, 2010), TP53 (Pakos et al., 2004), P-glycoprotein (Pakos and Ioannidis, 2003) et al.) and prognosis in patients with osteosarcoma. The results indicated that VEGF (Qu et al., 2012) and HER-2 (Li and Geng, 2010) were not significantly associated with prognosis in human osteosarcoma, whereas TP53 (Pakos et al., 2004) and P-glycoprotein (Pakos and Ioannidis, 2003) were significantly associated with prognosis in patients with osteosarcoma. However, besides our study, these meta-analyses consider Ezrin positivity was associated with a lower 2-year survival rate.

Several limitations of this meta-analysis must be acknowledged. First, publication bias may be a problem in meta-analyses. We tried to identify all relevant data and retrieve additional unpublished information, but some missing data was unavoidable. Typically, publication bias results in seeing stronger associations in smaller studies than in larger studies. However, in our meta-analysis, we reassuringly observed a stronger association of Ezrin positive status with 2-year mortality in larger studies. Thus, the association was clearer in high-quality studies with blinded assessment of outcomes. Secondly, there was some unavoidable variability in definitions of methods, measurements, and outcomes in each study, despite our effort to standarise definitions. Thirdly, the sample size of the meta-analysis is still modest, however, given that osteosarcomas are not very common on a population basis, the sample size of this investigation is one of the largest to date among studies targeting this malignancy.

The survival rate is only one of indicators which were used to evaluate the prognosis of patients. We also try to use other indicators such as metastasis. However, only two studies provide specific data of metastasis and the different definitions of metastasis are used in these two articles. Therefore, we had to abandon the analysis of indicator of metastasis. We also look forward to more research on the metastasis in patients with osteosarcoma be carried out.

To conclude, according to this meta-analysis, our findings suggest that expression change of Ezrin is an independent prognostic factor in patients with

osteosarcoma. But currently studies are still controversial in some aspects. For better analysis the relationship between Ezrin expression with the osteosarcoma. It’s necessary to improve the experimental methods and detection methods, and to clear a unified quantitative standard. The way of the mechanism of Ezrin express in osteosarcoma is not clear yet. With further research, Ezrin might become another target of the treatment of osteosarcoma.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (Grant No.81160323) and the Guangxi Innovative Program of Graduate Education (Grant No.YCSZ2012041).

References


