CD55 limits sensitivity to complement-dependent cytolysis triggered by heterologous expression of α-gal xenoantigen in colon tumor cells

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COLORECTAL CANCER is the third most commonly diagnosed cancer in males and the second in females (28, 57). Surgery is the mainstay of treatment in most cases of colon cancer. Adjuvant therapies, primarily chemotherapy and radiotherapy, have improved the survival rate and quality of life in colorectal cancer patients. However, the 5-yr survival rate remains at ~50%, which explains the growing interest in alternative therapies that have no serious side effects and that have the potential to eradicate residual tumors after conventional treatment. A deeper understanding of the interaction between the host immune system and malignant tumors has enabled the development of effective clinical strategies that improve immune responses against tumors. We seek to develop a therapeutic strategy that induces a specific immune response resulting in direct tumor cell killing, depending on the activation of the complement system.

The carbohydrate epitope Galα1-3Galβ1-4GlcNAc-R (α-gal) is recognized in New World monkey and nonprimate mammals species such as the pig and rat but not by humans (4, 8, 9, 11, 18, 33). On the other hand, large amounts of naturally occurring antibodies specifically against α-gal epitope are present in the serum of humans who are not immune compromised (13, 17). The binding of antibody to α-gal epitope activates the complement system and leads to the hyperacute rejection (HAR) of the α-gal-expressing cells and tissues (3, 10, 12, 15, 16, 32, 49).

α-(1,3)-Galactosyltransferase (α1,3GT) is responsible for the synthesis of the α-gal epitope. Humans lack this enzymatic activity due to the inactivation of the α1,3GT gene (38). The α1,3GT cDNA has been cloned from New World monkey (19), mouse (37), and bovines (29). It has been successfully expressed in a number of human cell lines (14, 24, 27, 53). The α-gal epitope increases the sensitivity of tumor cells to human complement-dependent cytolysis (CDC) in cell lines of human pancreatic cancer (1), hepatocellular carcinoma (66), and breast cancer (52). In some cases, however, the α-gal epitope did not increase the sensitivity of human tumor cells to human complement-mediated lysis (64). The significant differences in sensitivity to CDC among different tumor cell lines suggest that there is some underlying mechanism responsible for the sensitivity of α-gal-expressing tumor cells to α-gal-mediated lysis.

As an indispensable component of innate immunity, the complement cascade participates in host defense against infection and clearance of immune complexes (54, 69). The activation of the complement system is tightly controlled by many factors, and membrane-bound complement regulatory proteins (mCRPs) are important contributors to the control of complement attack. Many malignant tumor cells overexpress at least one of the mCRPs, including membrane cofactor (CD46), decay accelerating factor (CD55), and protectin (CD59), to avoid complement-dependent cytolysis (21, 22, 25, 36, 43, 56). As mentioned previously, one strategy that has been discussed is to engineer cancer cells to express the heterologous α-gal antigen. However, these studies did not explore the feasibility of α-gal-mediated cell lysis in colon tumors cells. In addition, research on the relationship between mCRP expression and the tumor cell sensitivity to α-gal-induced lysis is rare. In this study, we engineered human colorectal carcinoma cell lines to express the Banna minipig inbred-line (BMI) α1,3GT gene in an attempt to induce the direct destruction of those cells via complement activation and lysis. We also
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NHS for 1 h at 37°C and mixed with 0.4% trypan blue. Living and dead cells were counted. The survival rates were evaluated as follows: survival rate = living/(dead + living) × 100%, where “living” = no. of living cells, and “dead” = no. of dead cells.

Detection of mCRP expression by flow cytometry. Cells were removed from the culture flask using 3 mM EDTA, washed, and then incubated with appropriate FITC-labeled mAbs for 30 min at 4°C, washed, and then resuspended in formaldehyde fixing solution (1% BSA-PBS + 1% polyphosphate formaldehyde) and analyzed by flow cytometry (FCM).

Immunofluorescence assay of terminal complement complex (C5b-9) deposition. After incubation with 50% NHS, the cells collected with 3 mM EDTA were washed twice, fixed with 4% paraformaldehyde, and incubated with 1% bovine serum albumin (BSA) overnight at 4°C. This was followed by incubation with 100 μl anti-C5b-9 mAb, which was diluted with PBS containing 1% BSA (PBS-BSA) for 1 h at 37°C. After being washed with PBS, the cells were incubated with FITC-conjugated goat anti-mouse IgG antibodies for 30 min at room temperature and then incubated with 100 μl of 0.5 μg/ml DAPI. The cells were washed to remove unbound antibodies and DAPI. Cell suspensions (10 μl) were transferred to glass cover slips for confocal microscope.

Complement-mediated cytolyis assay of Ls-174T-GT cells after treatment with CD55 and CD59 blocking antibodies. The effect of CD55 and CD59 on complement susceptibility of α-gal-expressing Ls-174T cells was also evaluated by the cytolyis assay after blocking CD55 and CD59 function with special antibodies. Briefly, 1 × 10⁶ cells were incubated with 150 μl blocking antibodies (10 μg/ml) for 10 min at room temperature prior to the addition of NHS (50%). The number of dead and living cells was counted separately, and survival rate was calculated as above.

Downregulation of mCRP expression with shRNA. The targeting sequences for shRNA plasmids were as follows: CD55, TTAGCTACATCTACTCAGC (55); CD46, GGCATCTACGAGTTTGTGTTTATT; CD55, GCGCTTTCCTGGGTTGCT-3′; CD59, TGAGCTAAACACCTTTCCTGGT-3′. The negative control sequence (NC) was TTCTCCGAACGTGTCACGTT. The negative control sequence (NC) was TTCTCCGAACGTGTCACGTT. The target sequences for CD46, CD55, and NC were designed by Shanghai GenePharma. The targeting fragments were introduced into pGPU6/GFPNeo vector and designated shCD46, shCD55, shCD59, and shNC. The shRNA plasmids were transfected into Ls-174T-GT separately by Lipofectamine 2000 to knock down the expression of CD46, CD55, and CD59.

After transfection with shRNAs for 48 h, cytolyis assays were performed as described above. The interference efficiencies of shRNAs were determined through real-time quantitative PCR assay (RT-qPCR) and Western blotting analysis.

RNA extraction and RT-qPCR analysis. Total RNA was isolated from cells using Trizol reagent (Invitrogen) according to the manufacturer’s protocol. First-strand cDNA was synthesized by M-MLV reverse transcriptase (Promega) and oligo(dT). Following reverse transcription, real-time PCR was performed using SsoFast Eva Green Supermix (Bio-Rad) with the following primers: for human CD46, sense 5′-GGCCTTTCCTGGTGTCT-3′ and antisense 5′-ACGAGCAGGGGAGGAGTAC-3′; for human CD55, sense 5′-TTCGCCAGATGTTACATG-3′ and antisense 5′-TACAGTTAGTACCTCGGGAAACACT-3′; for human CD59, sense 5′-TTACCCAATGTGTTTGCACCAAA-3′ and antisense 5′-CTGGATTATGTCTCCTGCTGAC-3′; and for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sense 5′-AAAGGATGCCAGTGCTG-3′ and antisense 5′-GAGATGGTGATGGGGATTTCC-3′.

Western blotting analysis. Forty-eight hours after the transfection, cells were collected with PBS and lysed at 4°C in nonreducing cell lysis buffer. Each sample, containing an equal amount of whole cell lysates, was subjected to electrophoresis in 12% SDS-polyacrylamide gels, and then the protein was blotted onto a PVDF membrane. Primary antibodies against CD46, CD55, CD59, and GAPDH were used, according to the manufacturer’s recommendations. After washing the membrane, the
second antibody (HRP-conjugated anti-mouse IgG) was used for the detection of CD46, CD55, CD59, and GAPDH. The bands were visualized by the ECL detection system after washing the membrane.

C5a ELISA assay. Cells (1 x 10^6) from each group were collected with 3 mM EDTA, washed, and incubated with 50% pooled NHS for 1 h at 37°C. Further activation of the complement system was blocked by the addition of EDTA (10 mM final in supernatant). The supernatant was centrifuged at 1,000 rpm for 20 min to remove insoluble impurities and cell debris. The supernatant was collected and stored at −80°C until analysis. C5a were determined with a C5a ELISA kit (Elabscience). Measurements were performed in triplicate.

RESULTS

Establishment of the cell lines with stable expression of α-gal. Expression of heterogeneous α1,3GT and α-gal epitope in stably transfected cells LoVo-GT, SW620-GT, and Ls-174T-GT was identified by direct immunofluorescence staining (DIF). The α-gal epitope density on the membrane of stably transfected cells was examined through FCM. The results of direct immunofluorescence staining indicated that LoVo-GT, SW620-GT, and Ls-174T-GT showed a strong expression of α-gal similar to the positive control cell PIEC (Fig. 1). No FITC-BS-IB4 signal was detected on the membrane of their corresponding parental and α1-GT-transfected cell lines (data not shown). The FCM results also showed a strong expression of α-gal in LoVo-GT, SW620-GT, and Ls-174T-GT cells. Compared with LoVo-GT and Ls-174T-GT cells, SW620-GT had a relatively lower level of α-gal as revealed by the peak shift in the FCM results (Fig. 2) and the green fluorescent signal in DIF data (Fig. 1). The PIEC were used as a positive control.

Complement-mediated cytotoxicity assay. To investigate whether expression of α-gal on tumor cells would induce cell direct killing through CDC, the pooled normal human serum

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Fig. 1. The expression of epitope Galal-3Galβ1-4GlcNAc-R (α-gal) on stably transfected cells observed by direct immunofluorescence (×20). FITC-conjugated BS-IB4 lectin staining of the surface α-gal was performed following the selection of stably transfected cells. The α-gal was found to be expressed on LoVo-GT (B), SW620-GT (C), and Ls-174T-GT (D), as well as in the positive control pig iliac arterial endothelial cell line (PIEC) cells (A).

Fig. 2. Analysis of α-gal expression on the membrane of stably transfected cells. Strong expression of α-gal on stably transfected cells as detected by flow cytometry (FCM) and almost no expression on the corresponding parental cells. a: PIEC; b: LoVo; c: LoVo-GT; d: SW620; e: SW620-GT; f: Ls-174T; g: Ls-174T-GT.
(NHS) was used as the source of both complement and anti-\(\alpha\)-gal-specific antibodies. As shown in Fig. 3, the \(\alpha\)-gal-expressing Ls-174T exhibited similar survival rate as the parental and p1-GT-transfected cells. No significant cell killing was detected when the cells were exposed to 50% pooled NHS-containing media. However, \(\alpha\)-gal-mediated CDC was significantly increased in \(\alpha\)-gal-expressing Lovo and SW620 cells, and 99.8% of LoVo-GT and 97.8% of SW620-GT cells were killed in the same media. No cytolysis was observed in their corresponding parental and p1-GT transfected lines. No overt changes were observed when the \(\alpha\)-gal-expressing cells were exposed to the inactivated pooled normal human serum (INHS) compared with their corresponding control cells (data not shown). SW620-GT cells with a relatively weaker expression of \(\alpha\)-gal than Ls-174T-GT were extremely sensitive to CDC, suggesting that \(\alpha\)-gal epitope density is not the limiting factor in cell resistance to CDC. It also suggests that a specific mechanism is associated with \(\alpha\)-gal-expressing tumor cell sensitivity to NHS-mediated lysis.

**\(\alpha\)-gal-Mediated complement activation.** To determine whether the human complement cascade was activated by \(\alpha\)-gal, the cells were incubated with NHS. Subsequently the cells were stained for the presence of the membrane attack complex (MAC) on cell surfaces using an antibody against sC5b-9. The release of C5a in supernatant was also detected with a C5a ELISA kit. As shown in the Fig. 4A, the release of C5a was increased greatly in LoVo-GT and SW620 GT cells. By contrary, no differences in the released C5a were detected between the transfected and untransfected Ls-174T cells. All the parental cells showed a weak C5b-9 signal. Significantly stronger C5b-9 signals were detected on the LoVo-GT and SW620-GT cell membranes compared with the corresponding parental cells, but no difference in C5b-9 deposition was detected between Ls-174T-GT and its parental cells.

**Fig. 3. Trypan blue exclusion test:** cells from each group were incubated with 50% NHS, stained with 0.4% trypan blue. No significant difference was found between Ls-174T-GT cells and the Ls-174T parental control cells or Ls-174T-V. By contrast, the survival rate of LoVo-GT and SW620-GT decreased significantly compared with their parental cells, as almost all the \(\alpha\)-gal-expressing cells were killed in serum. **\(P < 0.05.**

**Fig. 4. Activation of complement mediated by \(\alpha\)-gal.** A: release of C5a. **\(P < 0.05.** B: deposition of C5b-9 (MAC). a: LoVo-GT; b: LoVo; c: SW620-GT; d: SW620; e: Ls-174T-GT; f: Ls-174T.
Expression of mCRPs detected by flow cytometry. Expression of the mCRP molecules CD46, CD55, and CD59 may protect tumor cells from anti-α-gal Ab-mediated lysis. The three colon tumor cell lines were evaluated for their surface mCRP expression levels by FCM (Fig. 5). The cell lines expressed different levels of surface CD46, CD55, and CD59. LoVo failed to express any of the three proteins. CD59 was strongly expressed by SW620 and Ls-174T, with only slight variation [mean fluorescence intensity (MFI) 51 ± 5 vs. 38 ± 8]. CD46 and CD55 varied between the two cell lines: CD46 in SW620 showed only half the intensity of CD46 in Ls-174T, as indicated by the MFI of FCM (231 ± 12 vs. 120 ± 24). Ls-174T showed a significant expression of CD55, while expression of CD55 in SW620 was not detected. Due to the fact that SW620 and Ls-174T cells showed high CD59 expression, SW620 expressed CD46 but were susceptible to lysis, and the Ls-174T cells, which were resistant to lysis, displayed significant expression of CD55 (Table 1), we speculated that the foremost mCRP that confers CDC resistance to cells was CD55. Based on the different intensities of CD46 expression between SW620 and Ls-174T cells, CD46 may play a secondary role in resistance to CDC.

α-gal-Expressing Ls-174-T-GT cells exhibit increased susceptibility to cytolysis when CD55 and CD59 are blocked. Blocking antibodies were used to evaluate the inhibitory effect of CD55 and CD59 on complement activation in Ls-174T-GT cells. As shown in Fig. 6, after incubation with the anti-CD55

Table 1. The effects of mCRP expression on cell sensitivity to α-gal-mediated CDC

<table>
<thead>
<tr>
<th>Cell</th>
<th>CD46, MFI</th>
<th>CD55, MFI</th>
<th>CD59, MFI</th>
<th>α-gal Mediated Cell Lysis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>LoVo</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>99.8 ± 0.1</td>
</tr>
<tr>
<td>SW620</td>
<td>120 ± 12</td>
<td>NA</td>
<td>38 ± 8</td>
<td>97.8 ± 1.38</td>
</tr>
<tr>
<td>Ls-174T</td>
<td>231 ± 12</td>
<td>27.78 ± 0.75</td>
<td>51 ± 5</td>
<td>8.01 ± 8.93</td>
</tr>
</tbody>
</table>

CDC, complement-dependent cytolysis; mCRP, membrane-bound complement regulatory protein; NA, undetectable; MFI, mean fluorescence intensity.

Fig. 5. Flow cytometry analysis of CD46, CD55, and CD59 expression on tumor cells. IC: Isotype control antibody. A: LoVo. B: SW620. C: Ls-174T.

Fig. 6. CD55 and CD59 blocking antibodies increase the susceptibility to cytolysis of Ls-174-T-GT cells. The survival rate of Ls-174-T-GT decreased with inhibition of CD55 or CD59 function. NHS, pooled normal human serum; INHS, inactivated pooled normal human serum; **P < 0.05.
antibodies, the cell survival rate decreased from 91.75 ± 2.9% to 34.95 ± 3.1%. Although anti-CD59 antibodies also enhanced complement-dependent cell killing (Fig. 6), the reduction in survival rate after blocking CD59 was significantly lower compared with that after blocking CD55 (P < 0.05). These data demonstrate that the presence of CD55 and CD59 may limit cell susceptibility to α-gal-mediated cytolysis, and that CD55 may be more important than CD59 in this regard.

**Downregulation of mCRP expression by shRNA.** Ls-174T-GT cells were transfected with shRNAs targeting CD46, CD55, and CD59 delivered by Lipofectamine 2000. After 48 h, knockdown efficiency was assessed by RT-qPCR and Western blotting. CD46 expression was inhibited by 76.43%, the CD55 expression by 63.74%, and CD59 expression by 72.26% (Fig. 7A). The decreased expression of mCRPs was confirmed by Western blotting analysis (Fig. 7B).

**Downregulation of mCRP efficiently increases sensitivity of the Ls-174T-GT to cytolysis.** After 48 h, shRNA plasmid-transfected Ls-174T-GT cells were collected and incubated with 50% NHS at 37°C for 1 h. Cell viability was significantly reduced and cell damage was significantly increased in shCD46 and shCD55 plasmid-transfected cells compared with the shNC group. Cell survival rate decreased from 89.95 ± 5.3% to 63.41 ± 6.13% in the shCD46 group, and 48.66 ± 3.7% in the shCD55 group. Cell lysis was not significantly increased in shCD59 plasmid-transfected cells compared with the parental cells (Fig. 8A). Complement activation was enhanced by silencing CD46 and CD55, as indicated by the significant increase in C5a release (Fig. 8B).

**DISCUSSION**

The human innate immune response to the xenoantigen α-gal epitopes may be exploited as a new approach for cancer therapy. The binding of anti-α-gal antibodies to α-gal-expressing tumor cells can induce the destruction of tumor cells through CDC, antibody-dependent cell-mediated cytotoxicity (ADCC), and enhanced tumor-specific adaptive immune re-
sponses. This has been confirmed in vitro as well as in α1,3GT knockout mice studies (7, 39, 40, 50, 51, 59, 61). In this study, we modified the colon tumor cell lines LoVo, SW620, and Ls-174T to express the α-gal antigen, and named them LoVo-GT, SW620-GT, and Ls-174T-GT, respectively. The α-gal-expressing LoVo and SW620 cells became sensitive to CDC when incubated with 50% pooled NHS-containing media. However, the Ls-174T-GT, which had a higher α-gal expression than SW620-GT, exhibited strong resistance to NHS-induced cytosis. The significant increase in released C5a and deposited C5b-9 in α-gal-expressing LoVo and SW620 compared with their corresponding parental cells demonstrated significant changes in complement activation in those α-gal-expressing cells. However, no increase in C5a and C5b-9 deposition was observed in Ls-174T-GT compared with Ls-174T, indicating that Ls-174T-GT cells were strongly resistant to CDC, induced by α-gal binding to the anti-α-gal antibodies present in human serum.

The increased C5a release and C5b-9 deposition was consistent with increased sensitivity to CDC. We speculated that the complement activation level was the main factor underlying differences in cell death among different α-gal-expressing cells. The activation of the complement cascade mediated by α-gal is affected by α-gal density on the targeted cells, blood type, anti-α-gal Ab titers, and concentration of complement factors (35, 41, 63, 67). In our study, the Ls-174T-GT, with a higher level of α-gal expression than SW620-GT, showed significantly lower sensitivity to CDC compared with SW620-GT. This finding indicates that α-gal density on the targeted cells is not the limiting factor in complement activation. Our use of pooled serum and pig iliac artery endothelial cell lines PIEC eliminated the influence of blood type, anti-α-gal Abs titers, and concentration of complement factors. The PIEC cells, which naturally express high levels of α-gal (46), were used as a positive control to assess the activity of anti-α-gal Abs and complement in cytolysis assays. The serum was used in a later experiment only if PIEC cells showed more than 99% cell death. We speculated that α-gal-expressing tumor sensitivity to NHS-mediated lysis is controlled by the factors that regulate the complement cascade.

We detected the expression of CD46, CD55, and CD59 by FCM. We found that the three colon cell lines expressed different levels of mCRPs. The most notable difference among the three cell lines was the expression of CD55: no CD55 expression was observed on LoVo and SW620, which were susceptible to α-gal-mediated CDC. However, significant CD55 expression was found on the Ls-174T cells, which exhibited strong resistance to α-gal-mediated CDC. The data indicated that CD55 expression has closest correlation with resistance.

To verify that CD55 expression on Ls-174T-GT tumor cells conferred resistance to α-gal-induced CDC, the function of CD55 was blocked with specific antibodies. We found that the blocking of CD55 rendered cells sensitive to CDC. Specific antibodies against CD59 were used to observe its role in Ls-174T-GT CDC resistance. The reduction in Ls-174T-GT cell survival rate following treatment with CD59-blocking antibodies was significantly less than the drop in survival rate when treated with CD55-blocking antibodies. To further investigate the impact of mCRP expression on sensitivity to α-gal-induced CDC, we silenced the expression of mCRPs with shRNA plasmids against CD46, CD55, and CD59 separately. Cell viability was significantly reduced and cell damage was significantly increased after the silencing of CD46 and CD55. However, no significant increase in Ls-174T-GT cell lysis was detected after downregulating CD59 expression with shRNA. The release of C5a increased greatly after the downregulation of CD46 and CD55, but C5a release did not increase when CD59 was silenced. This may be explained by the fact that the CD59 acts by preventing the incorporation of multiple copies of C9 on target cell membranes downstream of C5a release. However, the results of cell viability assays indicated that CD55, not CD59, was the most important mCRP affecting the sensitivity of α-gal-expressing colon tumor cells to CDC induced by natural anti-α-gal antibodies, and an abnormally high expression of CD46 may also be associated with colon cell resistance to α-gal-induced CDC. The relationship between CD46 and tumor cell resistance in α-gal-mediated CDC has not been previously reported. Our findings confirm that a fairly high level of CD46 limited cellular sensitivity to cytolysis.

The role of CD55 and CD59 in the resistance of α-gal-expressing tumor cells to α-gal-mediated CDC has been reported in several studies. Jager et al. (27) showed that α-gal-expressing human fibrosarcoma cells HT1080 became sensitive to CDC after the removal of CD55 and CD59 with nonhuman phosphatidylinositol-specific phospholipase C (PI-PLC). Hellrung et al. (24) modified A549 to coexpress α-gal epitope peptides and PI-PLC. The CD55 and CD59 on the A549 cells were effectively removed by the secreted PI-PLC. The A549 coexpressing α-gal and PI-PLC exhibited a fivefold increase in sensitivity to anti-α-gal-mediated complement lysis compared with the cells expressing α-gal alone (24). PI-PLC also removed other surface proteins along with CD55 and CD59 anchored to the membrane by the phosphatidylinositol (PI) moiety, thereby resulting in nonspecific membrane perturbations. Therefore, the relationship between enhanced sensitivity to CDC and reduced expression of CD55 and CD59 was not confirmed. In our research, however, we verified the function of each mCRP adequately by using blocking antibodies and RNA interference.

As our study shows, CD55 expression is the most important factor in conferring CDC resistance to α-gal expression colon cells in vitro. Since the activation of complement cascade may lead not only to CDC mediated cell death, but also plays an important role in the enhancement of the ADCC and the adaptive immune response via the release of complement activation products, such as C5a and C3a (6, 23, 31, 34, 48, 60). Thus we speculate that the expression of CD55 may further inhibit the antitumor immune response by affecting ADCC and the adaptive immune response in vivo. CD55 may be a key indicator of the effectiveness of α-gal-based therapeutic strategies in treating colon cancer. A better effect might be obtained when this therapy strategy is used in the colon tumor that failed to express CD55. Our results strongly support CD55 expression should be an important consideration when using α-gal in colon cancer therapy.

Other challenges need to be addressed before the α-gal-induced cytosis of tumor cells can be exploited in clinical application. An understanding of how to maintain stable and targeted expression of α-gal on tumor cells in situ will be required for the elimination of tumor cells by the immune system.
response against α-gal epitope. The development of more efficient gene transfer systems may enhance the effectiveness of this strategy. Selective introduction of α-gal into the tumor of a patient may be achieved by the use of receptor/ligand, transcriptional targeting, and oncolytic viruses (5, 30, 44, 47, 62). We believe that the use of α-gal as a target antigen to induce the complement cascade, in order to eliminate tumor cells, will become a potential future cancer treatment for humans. We expect better outcomes of α-gal immunotherapy will be achieved with the development of biotechnology and with a deeper understanding of the mechanism of CDC.

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REFERENCES


