REVIEW ARTICLE

Prostate cancer: The revolution of the fusion genes


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Abstract

Background: TMPRSS2-ETS fusion gene rearrangements constitute a very common and specific alteration in prostate cancer cells. These genetic alterations lead to the overexpression of ETS genes which encode the E26 family of transcription factors involved in cell proliferation. Of this family, the ERG oncogene is overexpressed in almost 50% of prostate cancer cases.

Evidence synthesis: TMPRSS2-ERG overexpresses ERG through an androgen-mediated response. Structurally, the rearrangement is due to interstitial deletion and to a lesser extent to reciprocal translocation and plays a key role in cellular metabolism. Almost all fusion gene transcripts produce a truncated ERG protein and the presence of a specific isoform of this gene suggests the clonality of the tumor; hence, metastasis shares the fusion gene status of their primary lesion. Although the prognostic implications of TMPRSS2-ERG have not been fully elucidated, they constitute a field of great diagnostic potential and, therefore, the development of techniques to identify and to analyze the presence and characteristics of this gene in a non-invasive fashion deserves great interest in this area. Currently, there is evidence supporting the hypothesis that the presence of fusion gene differentiates two molecular groups within prostate cancer with a differential behavior making the fusion gene a potential therapeutic target. In this regard, the use of anti-HDAC (trichostatin), antagonists of estrogen receptor alpha and abiraterone acetate have shown promising results.

Conclusions: This review describes the great potential offered by the investigation of fusion genes in PC and the need for further studies.

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Prostate cancer: The revolution of the fusion genes

Context

Prostate cancer (PCa) is the third most common tumor type in men. The appearance of this neoplasia is linked to age. In the European Union, PCa is directly responsible for the death of 3% of men and 10% of cancer deaths. The incidence of PCa has risen in recent years, primarily due to the significant increase in life expectancy, and secondly because of the introduction of the determination of serum PSA levels in PCa screening, raising the diagnostic in the preclinical phase. In Spain, the epidemiological situation of PCa is not significantly different from the rest of Europe. Every year, some 13,300 new cases are diagnosed (13.6% of tumors among Spanish men), with survival at 5 years around 65%, with an average age of death of 75 years.1

Histologically, PCa is constituted of a heterogeneous mixture of cells, mainly epithelial and stromal.2 This process begins with a dysplasia that starts as a proliferative inflammatory atrophy (PIA), progressing to prostatic intraepithelial neoplasia (PIN), and in some cases it leads to a carcinoma. There is evidence to suggest that one of the triggers of tumorogenesis could be a prostate inflammation due to infectious agents or ingestion of carcinogens. In parallel, some cells accumulate genetic alterations that, along with the androgenic signaling, stimulate the growth and proliferation of the tumor.3

Clinically, there are two large groups of PCa: prostate tumors able to spread that will end up being lethal, and others that are relatively indolent,4 which, to start with, raise the problem of how to distinguish some tumors from others and the manner of best clinical approach in each case. Currently, serum PSA levels provide highly organ-specific information, but little disease-specific. Thus, both in benign prostatic hyperplasia and prostatitis, serum increases of this biomarker are produced, but many patients with localized PCa also have PSA values that overlap with those of healthy subjects, resulting in a gray area of difficult interpretation of the range between 4 and 10ng/ml.4 Moreover, numerous studies suggest that PCa is overdiagnosed in 30–50% of the cases, that is, not all the patients with an elevated PSA have a prostate tumor. After the diagnosis, the main prognostic factor is the Gleason score, which consists of assigning a grade of 1–5 in descending differentiation to each of the two main foci of the tumor. The sum of both values is the score. Although this parameter is the gold standard in the clinical management of PCa, it presents certain problems: first, the determination is made on tissue obtained from a prostate biopsy, a surgical procedure that has certain comorbidities, particularly significant in elderly patients; besides, this score suffers from interpretive variation.5

In the prognosis of the disease, the lack of a reliable method capable of determining the time at which the prostate tumor will become hormone-resistant is problematic, because from here on, the patient’s prognosis worsens and bone metastases, for which currently only palliative treatment is available, often occur.6

For all this, it is very important to identify new biomarkers that represent useful tools in the diagnosis and clinical management of PCa. These markers should be determinable by objective, quantitative and mechanism-specific techniques, and as far as possible, they should be accessible by noninvasive methods.

This review aims to provide an overview of the status of some of these biomarkers, with particular emphasis on

Cáncer de próstata: la revolución de los genes de fusión

Resumen

Contexto: Los reordenamientos TMPRSS2-ETS constituyen una alteración específica y frecuente en tumores prostáticos que conlleva la sobreexpresión de los genes ETS que codifican para la familia E26 de factores de transcripción, promoviendo la proliferación celular. De entre estos ERG sobreexpresa en casi el 50% de los carcinomas prostáticos.

Síntesis de evidencia: TMPRSS2-ERG sobreexpresa a ERG en respuesta a andrógenos. Estructuralmente este reordenamiento se debe a una delección intersticial y, en menor medida, a una translocación recíproca, y tiene un papel clave en el metabolismo celular. Casi todos los transcritos del gen de fusión producen una proteína ERG truncada, y la presencia de una determinada isoforma de este gen indica la clonalidad del tumor, de modo que la metástasis comparte isoforma de TMPRSS2-ERG con su localización primaria. Aunque las implicaciones pronósticas de TMPRSS2-ERG no están totalmente elucidadas se considera un campo de gran potencial diagnóstico, por lo que el desarrollo de técnicas que permitan determinar la presencia y características de este gen de forma no invasiva es muy interesante. La presencia del gen de fusión constituye dos grupos moleculares dentro del CaP con un comportamiento evolutivo claramente diferencial, lo que hace que farmacológicamente el gen de fusión constituya una diana terapéutica potencial. En este sentido, el uso de fármacos anti-HDAC (tricostatina), antagonistas del receptor de estrógenos alfa y acetato de abiraterona han mostrado resultados prometedores.

Conclusiones: Esta revisión expone el gran potencial que representa la investigación de los genes de fusión en el CaP y la necesidad de profundizar en su estudio.

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New biomarkers in prostate cancer

One approach in the search for biomarkers is to study the expression of genes associated with PCa. Thus, underexpression of the GSTP1 gene (Glutathione S-transferase-Pl) (11q13) due to hypermethylation of its promoter region has been described. This gene catalyzes the glutathione-mediated molecular detoxification. However, given the size of the human genome and the low percentage of coding DNA, most chromosomal breaks that ultimately result in a balanced translocation would not produce a phenotypic effect because they do not affect coding regions.

Fusion genes have been extensively characterized in lymphomas, leukemias and sarcomas, but only recently have started to be identified and characterized in carcinomas, mainly in PCa although they are also being identified in papillary and follicular thyroid carcinomas, in some kidney and mucopidermoid carcinomas. The delay in the discovery of these genes in carcinomas is probably due to problems in the use of classical cytogenetic techniques in this type of tumors.

In the field of phenotypically significant fusions involved in cancer, we have two great groups, in which overexpression of an oncogene such as COL1A1-PDGf-beta, present in dermatofibrosarcoma protuberans, is produced; and the second group in which there is a chimeric protein that implies a transcription factor, such as EWS-FLI1 in Ewing’s sarcomas. While the presence of chimeric genes cannot be observed directly using techniques of high-output analysis (cDNA arrays), in data sets obtained in these expression experiments, gene rearrangements and copy number alterations can be shown, provided that non-traditional analytical treatments are used for data processing. Student’s t-test, for example, detects genes that constantly overexpress in a certain type of cancer, that is, they have a profile of biomarkers, but it is ineffective when it comes to revealing genes that overexpress in a subset of tumors within a given type of cancer (outliers).

In 2005, the journal Science published an article by M. Arul Chinnaian’s group, from Michigan University, in which a bioinformatic method called COP A (Cancer Outlier Profile Analysis) is implemented. This test seeks to find and reveal genes that overexpress in a subset of cases of a particular cancer (in this case PCa). With that aim, they analyzed data from 10,486 microarray experiments contained in the Oncomine Database (http://www.oncomine.org/). In developing this method is the idea that with the evaluation of variance, using the median instead of the mean, the peaks of expression of the genes that only overexpress in a subset of the data will be maintained. This analysis included genes with a known overexpression of a given type of cancer as a confirmation, for example RUNX1 in leukemias. ERG (21q22.3) and ETV1 (7p21.2) genes were those with a higher profile of outliers for PCa. Both genes are transcription factors of the ETS family (E26) involved in cell proliferation processes.

The discovery of TMPRSS2-ETS in prostate cancer

The fusion genes are formed when two chromosomes, or two regions of the same, break and change position leading sometimes to a new gene (also called chimeric gene) with a new function. These genes are the result of structural chromosome aberrations (reciprocal translocations, deletions or inversions) that have been described in many types of cancer. However, given the size of the human genome and the low percentage of coding DNA, most chromosomal breaks that ultimately result in a balanced translocation would not produce a phenotypic effect because they do not affect coding regions.

Gene fusion expression pattern of these transcription factors of the ETS family is mutually exclusive in a subset of PCa cases, a behavior common to other tumors such as Ewing’s sarcoma.
in which overexpression of these genes is related to EWS-STD gene fusions.\textsuperscript{20} From this analogy, Chinnaiyan’s group hypothesized that overexpression of ERG and ETV1 may be due to a gene fusion. In order to test the hypothesis, they used the technique of rapid amplification of cDNA ends (RLM-RACE) determining that the TMPRSS2 gene was fused in the 5’ position of these ETS family members.\textsuperscript{13}

**Evidence synthesis**

**Biological implications**

**TMPRSS2** is located in the 21q22.3 locus and encodes for a transmembrane receptor of the STP family (type II transmembrane serine protease) with a multimeric structure. This protein comprises a protease domain from S1 family, a LDLRA domain that forms a calcium-binding site, and a third transmembrane domain. TMPRSS2 is regulated by androgens, as it has an androgen response element in the promoter region of its gene; it is found in the seminal fluid proteome and is highly expressed in the prostatic tissue and, to a lesser extent, in the epithelial tissue of the colon, stomach, epididymis and breast. Functionally, by activating this protein, the serine-protease domain is released from the cell surface to the extracellular space and activates PAR-2 (protease-activated receptor), which, as mentioned above, is a G receptor that plays an important role in PCa metastasis.\textsuperscript{10} Nevertheless, its significance is unclear, as in TMPRSS2-knockout mouse models it showed a normal phenotype, suggesting that it is a redundant gene.\textsuperscript{21}

For its part, ERG (21q22.2) encodes for a nuclear protein of 363 residues that specifically binds to DNA in regions rich in purine and acts as a transcription factor.\textsuperscript{22} It consists of 17 exons spanning 300 kilobases and generating, at least, 9 isoforms via alternative splicing, 7 of which code for proteins. This gene is expressed in endothelial tissues, hematopoietic cells, kidney and genitourinary tract. Specifically in PCa, ERG is the most persistently overexpressed gene.\textsuperscript{13} Among the different ETS family members (which also include ETV4, ETV5, etc.), many are involved in invasiveness and metastasis processes.\textsuperscript{14} This gene family is characterized by the presence in all ETS members of DNA binding domains and several protein binding domains. Particularly, ERG interacts with histone H3 specific methyltransferase (ESET) and may participate in the epigenetic silencing of other genes.\textsuperscript{25} One hypothesis for the action of this gene in PCa would be that it fuse, a hormone-dependent activation of the transcription factor takes place. Therefore, this deregulation also affects these pathways of E-cadherin (CDH1), protein that is considered an important suppressor of metastasis. In PCa, the gene is underexpressed through hypermethylation of its promoter region.\textsuperscript{25} The involvement of the androgen receptor (AR), which acts as a master regulator of the progression of the transition from G1-S cell cycle phases by inducing signals that promote G1\textsuperscript{18} (Fig. 3), is also very important.

![Figure 1](http://www.elsevier.es) Performance of transcription factors of the ETS family on stress reactions mediated through MAPK signal transduction pathway. Adapted from Petrovics et al.\textsuperscript{23} The red background indicates overexpression of the gene and the blue one, underexpression. ERG highlighted in red.

Table 1 published, other two involving ETV4 and another ETV5, all with TMPRSS2 in position 5’. Fusions have also been characterized with other genes in 5’, such as SLC45A3 (Fig. 2). The latter translocations, although interesting from a biological point of view, have a rather limited clinical potential because of their low incidences (1–4%) (Table 1).

When it comes to proteins, almost every fusion gene transcript produces mainly a truncated ERG protein instead of a chimeric protein,\textsuperscript{13} since the translocated TMPRSS2 region corresponds to the non-coding promoter region of the gene. We have evidence that when TMPRSS2-ERG mRNA occurs in the primary tumor, the expression is lost when the androgen signaling of the patient is blocked, recovering it in the hormone-resistant tumor stage. When TMPRSS2 and ERG fuse, a hormone-dependent activation of the transcription factor takes place. Therefore, this deregulation also affects all the target genes of ERG, of which the most frequently co-overexpressed is HDAC-1, which catalyzes the histone deacetylation promoting gene expression.\textsuperscript{18}

As a whole, ERG overexpression may lead to alterations in the Wnt pathway (responsible for the cell proliferation mechanism), epigenetic reprogramming and deregulation of cell death pathways. It is worth stressing the role within these pathways of E-cadherin (CDH1), protein that is considered an important suppressor of metastasis. In PCa, the gene is underexpressed through hypermethylation of its promoter region.\textsuperscript{25} The involvement of the androgen receptor (AR), which acts as a master regulator of the progression of the transition from G1-S cell cycle phases by inducing signals that promote G1\textsuperscript{18} (Fig. 3), is also very important.
Figure 2  (A) Isoforms described to date of the TMPRSS2-ERG fusion gene that give an idea of the great instability of this rearrangement. (B) Other minority fusion genes involving different genes (usually hormone-regulated) in 5′ and other transcription factors of the ETS family in position 3′.

Table 1  Gene fusions described in CP that do not involve TMPRSS2 or ERG and are in the minority in terms of incidence, although of great interest in the elucidation of molecular mechanisms involved in the CP.

<table>
<thead>
<tr>
<th>Gene in 3′</th>
<th>Frequency</th>
<th>Bibliographical reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOXP1</td>
<td>ETV1</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>EST14</td>
<td>ETV1</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>HERVK17</td>
<td>ETV1</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>SLC45A3</td>
<td>ETV5</td>
<td>1.5%</td>
</tr>
<tr>
<td>HERV-K-22q11.23</td>
<td>ETV1</td>
<td>1%</td>
</tr>
<tr>
<td>SLC45A3</td>
<td>ETV1</td>
<td>1%</td>
</tr>
<tr>
<td>C15orf21</td>
<td>ETV1</td>
<td>1%</td>
</tr>
<tr>
<td>HNRN4B1</td>
<td>ETV1</td>
<td>1%</td>
</tr>
<tr>
<td>SLC45A3</td>
<td>ELK4</td>
<td>1%</td>
</tr>
<tr>
<td>NDRG1</td>
<td>ERG</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>KLK2</td>
<td>ETV4</td>
<td>&lt;1.4%</td>
</tr>
<tr>
<td>CANT1</td>
<td>ETV4</td>
<td>&lt;1.4%</td>
</tr>
</tbody>
</table>

Figure 3  Molecular mechanism of interaction of TMPRSS2-ERG with the signaling pathways of androgenic hormones characteristic of PCa, and more general ones in oncology such as Wnt and hedgehog.
### Table 2  Prognostic implications of the presence of TMPRSS2-ETS or features related with the gene.

<table>
<thead>
<tr>
<th>Biological characteristics</th>
<th>Method of detection</th>
<th>Number of cases</th>
<th>Prognostic implication</th>
<th>Bibliographical reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMPRSS2-ETS presence</td>
<td>RT-PCR</td>
<td>111</td>
<td>Metastasis and death due to PCa</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>61</td>
<td>Early recurrence after radical prostatectomy</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>102</td>
<td>Aggressive PCa</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>165</td>
<td>Aggressive PCa</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>26</td>
<td>Recurrence after radical prostatectomy</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Nested RT-PCR</td>
<td>55</td>
<td>Unrelated to the aggressiveness of PCa</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>63</td>
<td>Unrelated to the aggressiveness of PCa</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>50</td>
<td>Lower Gleason score</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>226</td>
<td>Classification in two PCa groups with different prognostic characteristics</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>96</td>
<td>Aggressive PCa</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>118 PCa 18 metastases</td>
<td>Aggressive PCa</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>521 PCa 40 metastases</td>
<td>Unrelated to the aggressiveness of PCa</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>15</td>
<td>Unrelated to the aggressiveness of PCa</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>196</td>
<td>Lower Gleason score</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>521 PCa 40 metastases</td>
<td>Lower Gleason score</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>RT-PCR FISH</td>
<td>82</td>
<td>Unrelated to the aggressiveness of PCa</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>19 Xenotransplantations 7 PCa cell line 49 PCa</td>
<td>Greater recurrence-free survival</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>FISH</td>
<td>445</td>
<td>Lower overall survival</td>
<td>18</td>
</tr>
<tr>
<td>TMPRSS2-ERG deletion and with two or more copies of the fusion gene</td>
<td>RT-PCR</td>
<td>114</td>
<td>Disease-free survival after radical prostatectomy</td>
<td>23</td>
</tr>
<tr>
<td>Overexpression of ERG</td>
<td>RT-PCR</td>
<td>114</td>
<td>Disease-free survival after radical prostatectomy</td>
<td>23</td>
</tr>
</tbody>
</table>
There is also a strong cooperativity between the presence of the fusion gene and the PI3K pathway, which plays a fundamental role in the metabolism of cancer. Firstly, the correlation between the presence of TMPRSS2-ERG, deletions in PTEN, and expression of MYC has been proven, and secondly, transgenic mouse models for the fusion gene develop PIN, but only in the context of a generalized activation of PI3K pathway.\(^{29}\)

**Clinical significance**

The discovery of this fusion gene family has several sides of particular interest: one may help to functionally elucidate the hormone-dependent behavior of PCa, and another may be a therapeutic target\(^{13}\); besides, it produces interesting diagnostic and prognostic possibilities.

While there are many fusions involving TMPRSS2 and other ETS family members, in future, TMPRSS2-ERG alone will be checked, since it is the most prevalent and the most extensively studied chimeric gene.\(^{13}\)

TMPRSS2-ERG has a frequency of 40–70% in PCa, which gives an idea of its potential as a biomarker.\(^{13,30}\) Although there are many rearrangements involving both genes, most of them involve the TMPRSS2 exon 1 and ERG (T1E4) 4 followed by the fusion of exons TMPRSS2 1 with ERG (T1E2) 2, which, on the whole, represent approximately 80% of these rearrangements.\(^{30}\) The expression of the protein encoded by the isoform T1E4 might promote tumor growth.

Prostate carcinogenesis is usually a multicenter process in which several pathogenic pathways co-exist with or without the concurrence of the ETS pathway.\(^{18}\) The multifocal PCa is a heterogeneous group of diseases originated in multiple independent clonal expansions. One way to determine the clonality of PCa is to establish the presence and isoform of TMPRSS2-ERG in the different foci of the tumor and metastasis. Foci were found in the same prostate with different isoforms of the fusion gene or without having it, and even two isoforms in the same focus. However, the metastasis always shares the status of the fusion gene with its primary tumor, indicating that it is a single clone that evolves from the original location for metastasis.\(^{18}\)

At diagnostic level, the potential of TMPRSS2-ERG detection is undeniable, given its specificity. While the presence of the fusion gene in frozen surgical pieces\(^{19}\) and in formalin-fixed paraffin embedded (FFPE) pieces\(^{30}\) is perfectly established, the determination in other less invasive samples such as blood\(^{31}\) and urine\(^{13}\) has an obvious interest.

There is still no consensus on the prognostic implications of TMPRSS2-ERG\(^{16}\) (Table 2). This may be due to the heterogeneity of the studied series and the different techniques used in the determination: fluorescent in situ hybridization and RT-PCR mainly.

There is plenty of evidence that seems to point to the fact that PCa with the TMPRSS2-ERG gene, and those that do not contain it, are two distinct groups in the disease. On the one hand, a study of gene expression revealed that tumors with the fusion gene have different transcriptomic profile. Specifically, 87 genes involved in the signaling pathway of estrogen hormones are overexpressed in positive TMPRSS2-ERG tumors with respect to cases that do not carry this gene.\(^{32}\)

Our group studied the clinicopathologic factors using multivariate analysis, stratifying the population according to the presence or absence of the TMPRSS2-ERG gene. The cases with the fusion gene had, as independent prognostic factors, the serum PSA levels at diagnosis, the Gleason score in the prostatectomy piece and the surgical margins, whereas non-rearranged cases showed prognostic value: the cT, the Gleason score and margins,\(^{30}\) which opens the possibility of considering optimized treatments depending on the status of the fusion gene.

The third argument of molecular stratification of PCa is the geographic distribution of genetic abnormalities. The eastern population has a lower incidence of PCa than Westerners. Mao et al. have recently reported that this difference in incidence entails gene differences. While the frequency of TMPRSS2-ERG rearrangement is around 50% of the cases in Westeners, in the Chinese population it would be 2.5%, and the deletions of the PTEN gene with a frequency of about 40% in the western population would only have an incidence of 7.6% in Chinese people.\(^{33}\)

TMPRSS2: ERG is also an interesting therapeutic target. In cell lines containing the rearranged gene, therapy with HDAC inhibitors (trichostatin) drastically reduces tumor growth.\(^{34}\) In addition, these same TMPRSS2-ERG + cases seem to respond well to estrogen receptor (ER) α agonist drugs and ER β agonist ones.\(^{13}\) Finally, and in clinical trials, the abiraterone acetate, a molecule that blocks androgen signaling inhibiting the cytochrome P17, has obtained promising results in TMPRSS2-ERG cases.\(^{13}\)

**Conclusions**

The high incidence and the connection with the androgenic signaling pathway of TMPRSS2-ERG make it a biomarker with much potential at a diagnostic and prognostic level, but much work is still necessary.\(^{13,18,23,30,35-40}\) Translationally, it is a clear therapeutic target.

Of great interest is also to continue investigating the involvement of the fusion gene in signaling pathways (androgen, PI3K, Wnt, Hedgehog), which may help to elucidate the still mysterious molecular biology of this type of tumors.

Finally, the detection of this anomaly in a minimally invasive way is promising. In summary, in cancer molecular biology, the field of fusion genes is promising not only at a basic and clinical but also at a translational level as well.

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**Conflict of interest**

The authors have no conflicts of interest to declare.
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References


