Clinica Chimica Acta 498 (2019) 52–61

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

Glycosylation products in prostate diseases

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ARTICLE INFO

Keywords:
Diagnosis
Glycans
Glycosylation
Lectins
Prostate cancer
Prostate specific antigen

ABSTRACT

Although prostate cancer is notable for its high incidence and mortality in men worldwide, its identification remains a challenge. Biomarkers have been useful tools for the specific detection of prostate cancer. Unfortunately, benign prostate diseases cause similar alterations in screening assays thus reducing the potential for early and specific diagnosis. Changes in glycan and glycoprotein expression have often been associated with the onset and progression of cancer. Abnormal glycans and glycoproteins have been reported as new biomarkers of prostate metabolism that can distinguish benign prostate disease and cancer in non-aggressive and aggressive stages. Carbohydrate-binding proteins known as lectins have been valuable tools to detect these changes, investigate potential biomarkers and improve our understanding aberrant glycosylation in cancer. Here we review progress in elucidating prostate disease and discuss the roles of glycans in the differential detection of benign and cancerous prostate disease. We also summarize the lectin-based tools for detecting glycosylation changes.

1. Introduction

Currently, thousands of people worldwide are diagnosed with various types of cancer. Cancer is a group of diseases characterized by the growth and multiplication of abnormal cells, and if not controlled, can easily lead to death. Among the cancers that affect the male population, prostate cancer is the second most common, surpassed only by lung cancer [1], and the second most frequent cause of cancer death in men in the United States, according to estimates by the American Cancer Society [2].

The latest global estimate also pointed to prostate cancer (PCa) as the second most common cancer in men, with about 13.5% of incidence in 2018. Incidence rates of this cancer remain high in the world's highest income regions, including North America, Western and Northern Europe, and Australia-New Zealand, while mortality rates from PCa tend to be higher in low average income areas, and parts of South America, the Caribbean as well as Sub-Saharan Africa. The very high incidence rates observed in developed countries are partly due to the widespread dissemination of prostate-specific antigen (PSA) screening and the high longevity of the population in those countries [3].

Cancer mortality can be reduced by early diagnosis and treatment. PCa screening and diagnosis is based on the PSA test and clinical evaluation by digital rectal exam. When abnormalities suggestive of cancer are identified, the diagnosis is confirmed by prostate biopsy. PSA is a glycoprotein synthesized by prostatic cells, considered the prostate tumor marker. Normally, this is secreted into the blood at very low concentrations ($0 \leq 4\,ng\,mL^{-1}$), however it can increase and reach quite high concentrations in individuals with prostate cancer [4]. The use of the PSA test for prostate cancer diagnosis is controversial, since benign prostate diseases, such as benign prostatic hyperplasia (BPH) and prostatitis can also be associated with increased serum PSA levels ($> 4\,ng\,mL^{-1}$), while a significant incidence of cancer has also been reported at reduced levels of PSA [4], hindering the diagnosis of prostate cancer and benign prostate diseases. Thus, many cases are sent unnecessarily for biopsy, highlighting the poor sensitivity and

Abbreviations: AQT, alpha-1-antichymotrypsin; Asn, asparagine; BPH, Benign prostatic hyperplasia; BPSA, Benign PSA; cPSA, complexed PSA; DRE, Digital rectal exam; EIS, Electrochemical impedance spectroscopy; FDA, Food and Drug Administration; fPSA, free circulating PSA; IARC, International Agency for Research on Cancer; KLK, Human kallikrein; LTA, *Lotus tetragonolobus* agglutinin; MAA, *Maackia amurensis* agglutinin; PCa, Prostate cancer; PSA, Prostate specific antigen; Ser, serine; SNA, *Sambucus nigra* agglutinin; Thr, threonine; TRUS, Transrectal ultrasound; WHO, World Health Organization

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https://doi.org/10.1016/j.cca.2019.08.003
Received 20 May 2019; Received in revised form 6 August 2019; Accepted 6 August 2019
Available online 07 August 2019

specificity of the PSA test for the diagnosis of the disease [5]. Recent research has focused on the development of alternative methods to identify new, more sensitive and specific markers for prostate cancer.

Methods based on glycosylation pattern analysis represent new approaches for the detection of several types of cancer, including prostate cancer. Changes in the glycosylation of free glycoproteins and on cell surfaces are often observed in cancer progression, resulting in specific glycosylation profiles that can be identified, quantified and used as cancer biomarkers [6]. PSA, for example, is found in the bloodstream in different forms and patterns of glycosylation, which commonly change in prostate cancer [7]. In addition to PSA, other glycoproteins derived from prostatic tissue, serum and seminal fluid from individuals with prostatic tumors have revealed significant changes in the glycosylation pattern that allow distinguishing between BPH and prostate cancer [8,9].

Lectins are natural and non-immune proteins with carbohydrate-binding sites, interacting with carbohydrates in a specific and reversible way. They have been widely used in the development of diagnostic methods to investigate disease-related glycosylation patterns, which can detect glycosylation alterations in circulating and cell-surface glycoproteins and assist in cancer diagnosis in the early stages of development [10,11]. Studies of glycosylation patterns in serum glycoproteins have employed highly accurate and sensitive techniques coupled to lectins, such as affinity chromatography, mass spectrometry, histochemistry, enzymatic immunoassays, point-of-care testing and biosensors [12,13].

In this review, we highlight advancements in the diagnosis of prostate diseases related to biomarkers, focusing on glycosylation changes and their role in identifying potential new targets based on altered glycans and glycoproteins. The differential detection of benign and malignant prostate diseases may be obtained by profiling these biomarkers. We also discuss the role and progress of lectin-based analysis for detection of glycosylation changes.

2. Prostate diseases

2.1. Benign

Benign diseases of the prostate represent an important health problem mainly for aging men through their associated symptoms and complications. The prostate is a walnut-sized gland that is part of the male reproductive system, located in the lower abdomen, below the bladder and surrounding the urethra at the neck of the bladder. This gland produces a fluid that nourishes and protects the sperm contained in the semen and is composed primarily of acid phosphatase, citric acid, fibrinolysin, PSA, proteolytic enzymes, and zinc. While size of the prostate is approximately that of a ping-pong ball, it may be much larger in elderly men due to a benign growth of the gland that occurs in most men from adulthood [14]. Prostatitis and benign prostatic hyperplasia or enlarged prostate, are benign diseases that affect the prostate [15].

Prostatitis is a commonly painful condition associated with bacterial infection or inflammation of the prostate gland (Fig. 1), which can affect men of any age, most commonly men younger than age 50. This condition affects about two million patients each year in the United States. Four types of prostatitis have been identified: chronic prostatitis or chronic pelvic pain syndrome (CPPS), acute bacterial prostatitis, chronic bacterial prostatitis and asymptomatic inflammatory prostatitis. The symptoms can vary depending on the cause and individual factors [16,17].

The symptoms of CPPS can involve pain or discomfort in some areas including the lower abdomen, between the scrotum and anus, the penis and the lower back; pain during or after ejaculation and urination; alteration in urinary frequency and a weak or interrupted urine stream [18]. The symptoms of acute bacterial prostatitis are severe and require immediate medical care. These include urinary frequency and urgency; nocturia; fever; pain in the genital area, lower abdomen and during urination; urinary blockage; nausea; vomiting and urinary tract infection. In the case of chronic bacterial prostatitis, the symptoms are similar to acute bacterial prostatitis; however, it is not severe and generally occurs after treatment of acute bacterial prostatitis and urinary tract infection [19]. The diagnoses of prostatitis are based on personal and family medical history, a physical exam such as digital rectal exam, and laboratory tests such as urinalysis, semen analysis, blood tests, transrectal ultrasound and biopsy. The treatment can vary according to prostatitis type, and includes antibiotics, anti-inflammatory drugs and other medications to decrease the symptoms [16,20]. In asymptomatic inflammatory prostatitis, men do not show symptoms, with the diagnoses obtained through testing for other urinary tract or reproductive disorders; this condition does not require treatment.

Benign prostatic hyperplasia (BPH) is a non-cancerous condition commonly associated with aging, which is more prevalent in men older than 50, reaching more than half of the male population in the seventh decade of life and almost all in the eighth decade [21,22]. This condition is characterized by benign enlargement of the prostate gland resulting from progressive hyperplasia that begins around age 25 and continues during the man's life (Fig. 1). Evidence suggests that the increase in levels of androgen hormones and estrogen within the prostate associated with aging men induces prostate cell growth and tissue hyperplasia, leading to obstruction of the bladder neck or urethra, making it difficult to urinate and causing incomplete emptying of the bladder with urinary retention [21,23]. Symptoms suggestive of BPH are urinary frequency and urgency, nocturia, a weak or an interrupted urine stream, urinary retention, difficulties to start a urine stream, urinary incontinence and pain during ejaculation and urination.

Some conditions are related as predisposing factors for BPH development, like age of 40 years and older, family history, obesity and circulatory diseases [24]. Treatment includes medication to decrease symptoms or surgical therapy when the symptoms are severe. Evaluation of the patient with BPH is performed through anamnesis with application of the prostate symptom score, physical analysis with digital rectal exam, laboratory evaluation through serum PSA, urinalysis and renal function, as well as imaging and urodynamics [23,25].

2.2. Cancerous

More than any other type, PCa is considered a cancer of senior citizens, occurring mainly in elderly men. About 62% of the world's diagnosed cases occur in men aged 65 years and older. Another risk factor to be considered is ethnicity, since PCa is approximately twice as frequent in Afro-descendants as in men of other races [26]. Genetic studies suggest that family predisposition may account for 5–10% of cases. Having a father or sibling with PCa, especially before 60 years of age, increases the chance of men developing the disease by more than twice. The risk is higher for men with several affected relatives, particularly if their relatives were young when cancer was found [27–29]. Other risk factors are investigated in relation to this disease, such as genetic mutation, diet, obesity, smoking, inflammation in the prostate, sexually transmitted infections and vasectomy [29,30].

Most PCa originate in prostate glandular cells, called adenocarcinoma; some can grow and spread quickly to other organs, but most grow slowly. Autopsy studies show that many elderly men and some young men who died of other diseases had PCa which never affected them during their lives. In its early stages, PCa usually causes no symptoms, a fact that makes early detection of the disease difficult, especially in individuals who do not perform screening tests. In more advanced disease, tumor growth often presses and obstructs the urethra (Fig. 2), causing the characteristic symptoms: weak or disrupted urine flow; inability to urinate or difficulty controlling urine flow; urinary frequency and urgency, especially at night; presence of blood in the urine and pain or burning on urination [31]. Being in an advanced state, PCa can spread through the body, reaching other organs and

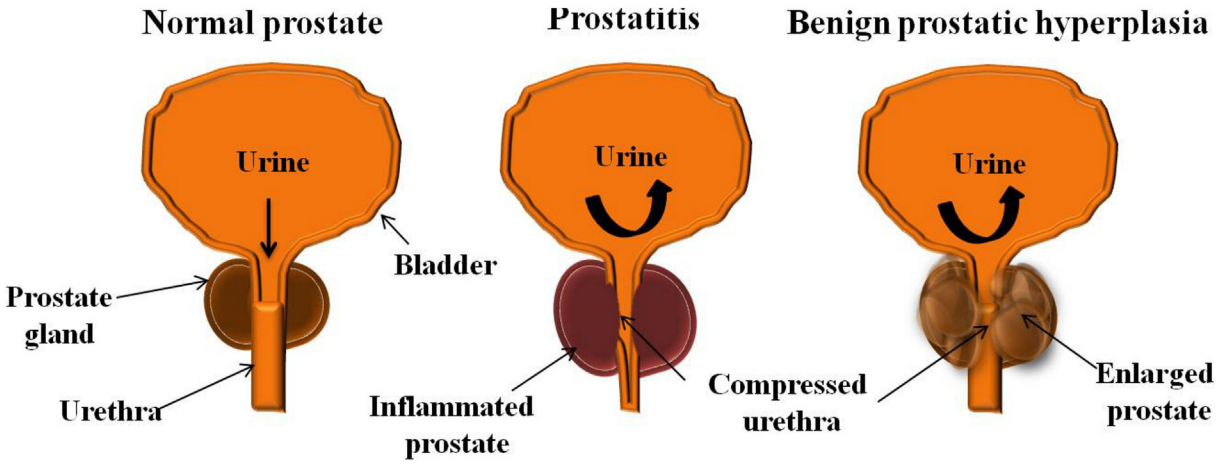

Fig. 1. Representation of normal prostate gland, prostatitis and benign prostatic hyperplasia.

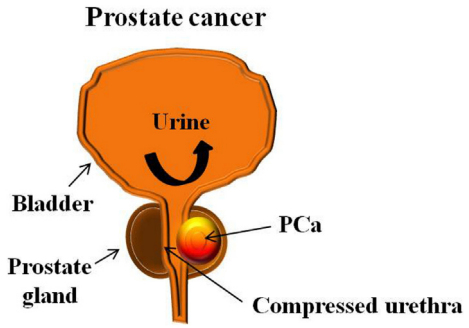

Fig. 2. Prostate gland with cancer tumor, PCa, compressing the urethra.

especially bones, including the spine, hip, ribs, femur, among other areas. Spinal pain in an individual at the age of risk or spontaneous fracture of the femur without any trauma may be caused by spread of the tumor [32].

2.2.1. PCa diagnosis

The diagnosis of PCa is made through screening by digital rectal exam and blood PSA test on an annual basis, beginning at age 50. In men belonging to risk groups (Afro- descendants or a relative diagnosed with PCa before the age of 65), the screening should begin at age 45, and at age 40 in men with several relatives already diagnosed with PCa at an early age [2,32].

PSA is a glycoprotein produced in prostate tissue and secreted into seminal fluid, being also found in serum and urine. The PSA test is routinely utilized as a prostate tumor marker for PCa screening. Most of the PSA produced is present in the seminal fluid at relatively high concentrations (0.5–5 mg mL^{-1}) and only a small fraction is released into the bloodstream, where the concentration varies from 0 to 4 ng mL^{-1} [33]. In healthy subjects, serum PSA levels are commonly $\leq$ 0.1 ng mL^{-1} and are considered normal until 4 ng mL^{-1}. However, any alteration in prostate gland architecture allows for higher concentrations of PSA to enter circulation. Values above 10 ng mL^{-1} suggest strong evidence of cancer; values between 4 and 10 ng mL^{-1} are classified into the diagnostic gray-zone category, not allowing a clear differentiation between PCa and other pathologies. Thus, biopsy is indicated for PSA levels above 4 ng mL^{-1} [34].

Although the PSA test revolutionized cancer testing since its first approval in 1986 by the US Food and Drug Administration (FDA), its clinical specificity for detecting PCa is questionable. Other prostate diseases such as BPH and prostatitis also cause increased PSA in circulation, and there are even cases of PCa that occur when PSA levels are considered normal [35]. Studies have reported a risk of 6.6–26.9% of PCa incidence when PSA levels are $\leq$ 4 ng mL^{-1} [35]. These factors confirm the limitations in terms of sensitivity and specificity of the PSA test for PCa diagnosis. Considering a cutoff point at 4.0 ng mL^{-1}, the PSA test presents an estimated sensitivity of 71% and a specificity of 46% [36]. Studies estimate that its positive predictive value is around 28%, which means that about 72% of patients with altered PSA are submitted to unnecessary biopsies [33,36].

When total PSA is below 4 ng mL^{-1}, or between 4 and 10 ng mL^{-1}, it is recommended to consider the free/total PSA ratio, which is inversely proportional to prostate and tumor volume, and the Gleason score. Cutoff values can vary with age range, being considered values of 10–25%. Results below cutoff values are considered suggestive of PCa, and above are suggestive of BPH [36,37]. Thus, the % free/total PSA ratio has shown greater sensitivity and specificity when compared with the PSA test and can decrease the number of unnecessary biopsies [38].

Studies also indicate that PSA expression is not tissue specific. The antigen has already been detected by immunohistochemistry in the endometrium; secretions from male periurethral and anal glands, breast tumors, lung adenocarcinoma, among other tumors [39]. Considering this statement and the other factors mentioned, it is important to associate the PSA test with other assays, such as digital rectal exam, for cancer detection.

The digital rectal exam (DRE) has been recommended because of the increased possibility of PCa diagnosis, especially in patients with normal levels of PSA. The presence of alterations observed by DRE is a strong indication for biopsy. Studies show that up to a fifth of patients with PCa show alterations only in the DRE, and among these, a third have PCa, even with normal PSA [36,40]. DRE sensitivity ranges from 48% to 59% and specificity from 89 to 92%. Positive predictive value is estimated between 28% and 40%. Despite the recommendation to perform the examination at each visit, the adherence to clinical practice of DRE is about 20%, which is considered low [36,40,41]. If there are abnormalities in digital rectal exam or PSA levels are equal to or > 4 ng mL^{-1}, or both, a histopathological exam of tissue obtained through prostate biopsy is indicated [36].

Transrectal ultrasound-guided (TRUS) prostate biopsy is the indicated method to confirm diagnoses of PCa when the patient shows abnormal PSA levels and/or a palpable alteration through DRE. This procedure involves insertion of an ultrasound probe about the size of a finger into the rectum, followed by biopsy with a spring-driven needle core biopsy device, or biopsy gun, for obtaining prostatic tissue for histological evaluation. The prostate cells collected by biopsy are analyzed and compared to normal prostate cells. The higher the degrees of abnormality found in prostate biopsy cells in relation to normal cells, the more aggressive is the cancer, and its spread will be faster. Following histological analysis, PCa is graded using the modified

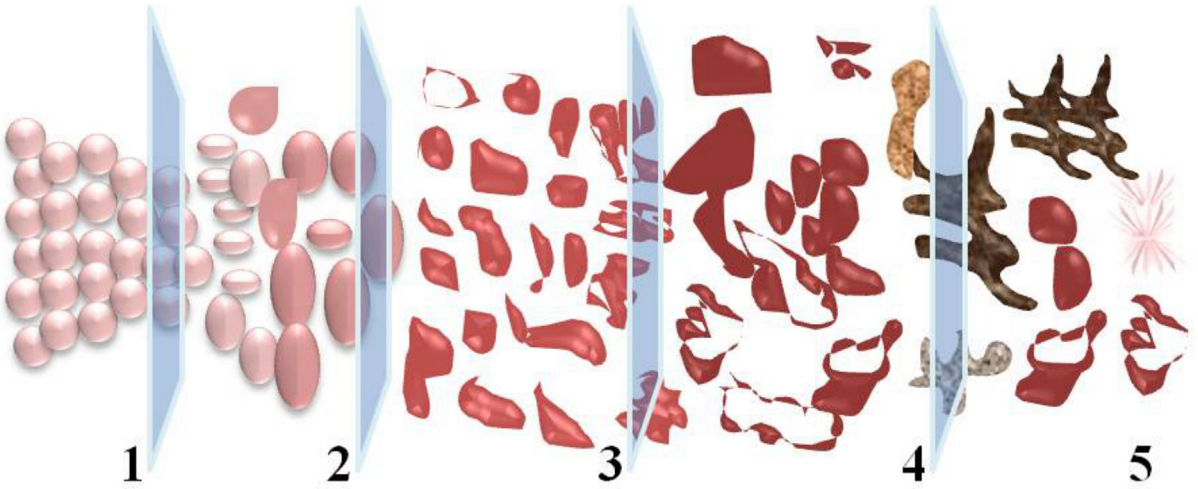

Fig. 3. Representation of prostate cancer cells in the Gleason grading system.

Gleason system.

The Gleason system [42] describes the degree of tumor aggressiveness [35]. Scoring of the Gleason system scale is based on histological patterns ranging from grades 1 to 5 (Fig. 3), with grade 1 being the least aggressive form and grade 5 representing the anaplastic tumor. To obtain the total Gleason score the two most frequent areas of the tumor are graded from 1 to 5 and the results are added, ranging from 2 to 10. The lower the score, the less aggressive the tumor is and the better the patient's prognosis, and vice versa. However, the evolution of histological diagnosis and treatment of PCa led to revisions of the Gleason system, which resulted in some modifications. This more recent classification does not assign scores from 2 to 5, and some patterns previously classified as score 6 are now classified as 7, and the tumors are classified into three levels: 6, 7, and 8–10.

Despite these changes, the Gleason system has some deficiencies, such as the final classification does not recognize that the tumors 3 + 4 and 4 + 3 are grade 7, as well as 8 and 9–10 scores show very different prognoses. Another deficiency is the lowest score to be considered is equal to 6, although the scale starts at grade 2, suggesting the cancer is more aggressive than it is, and arousing expectations and fear of the diagnosis by patients [43]. In 2013, a new Gleason classification system was proposed in an attempt to minimize these deficiencies. It consisted of five separate groups in grades from 1 to 5: the grade 1 group (Gleason score < 6), grade 2 group (Gleason grade $3 + 4 = 7$), grade 3 group (Gleason grade $4 + 3 = 7$), grade 4 group (Gleason grade 8); and grade 5 group (Gleason Degrees 9–10) [43]. In this way, more suitable classification and treatments can be obtained, mainly of low-grade PCa.

Despite allowing for definitive diagnosis of PCa, studies have shown that in up to a third of cases, cancer is not detected in the initial biopsy [44]. When the first biopsy is negative, a second biopsy is recommended, which when negative also reduces the possibility of development and diagnosis of PCa [41].

3. PSA biochemistry

PSA (or human kallikrein 3; KLK3) is a 237-amino acid serine protease with chymotrypsin-like activity, androgen regulation, and belongs to the kallikrein family. In contrast to the rodent tissue kallikrein (KLK) gene families, the human KLK gene family seems to consist of just three genes: KLK1 (encoding tissue kallikrein), KLK2 (encoding glandular kallikrein), and KLK3 (encoding PSA), which are encoded by a cluster of genes located in a region of 300 kb on human chromosome 19q13.4 [45–48]. PSA is synthesized by the prostate ductal and acinar epithelium, being initially translated as a prepropolypeptide with a 17-amino acid leader sequence that is cotranslationally cleaved during passage through the secretory pathway, producing an enzymatically inactive protein precursor (proPSA with 244 amino acids) named [−7]proPSA. This precursor contains an extra 24 amino acids that are activated by other kallikreins (hK2 or hK4), which cleave proPSA between the arginine at position 7 and the isoleucine at position 8, such that the N-terminal amino acid of the mature active PSA protein is isoleucine, yielding a 28–32 kDa mature active protease [49,50] consisting of five intrachain disulphide bonds and a single *N*-linked glycan [8,51–53].

PSA is highly abundant in the seminal fluid, present at concentrations of 0.5 mg ml^{-1} and greater [48,54], synthesized by both healthy and diseased prostate tissue which cleaves the proteins semenogelin I and II, which leads to the liquefaction of the semen [55].

It is important to note that truncated forms of proPSA could be also generated by cleavage. Isoforms with leader peptide variants of one, two, four, and five amino acids, named [−1]proPSA, [−2]proPSA, [−4]proPSA, and [−5]proPSA, respectively, have been identified [56]. Still in the lumen, mature active PSA is cleaved at specific sites, generating the BPSA isoforms (known as benign PSA) and iPSA (inactive PSA). All of these PSA isoforms are found as free circulating PSA (fPSA), which corresponds to 16% of total PSA [60]. Most of the circulating PSA corresponds to complexed PSA (cPSA) bound to protease inhibitors, mainly alpha-1-antichymotrypsin (AQT) and alpha-2-macroglobulin [57]. Fig. 4 represents PSA and the biosynthesis of its isoforms.

ProPSA subforms have been identified as the predominant constituents of fPSA. Several studies have shown the specificity of proPSA for detection of prostate cancer, especially [−2]proPSA and [−4]proPSA. Increased expression of [−2]proPSA has been associated with increased cancer aggressiveness and tends to accumulate in the serum of men with PCa [58]. Benign PSA (BPSA) is a distinct, inactive form of PSA that is prone to internal degradation at amino acid sites Arg85-Phe86, Lys145-Lys146, and Lys182-Ser183 (Fig. 5). BPSA is predominantly found in the prostate transition zone of men with BPH; in addition, it is thought to arise from post-translational cleavage by specific proteases in hyperplastic BPH tissue [59].

The emergence of the importance of PSA for the detection of prostate cancer and its potential use as a biomarker for residual or recurrent disease has been reported [60]. Those authors showed that patients with PCa had high serum levels of PSA; in addition, they observed that PSA increased with advanced clinical disease, proportional to tumor volume, and its levels in serum reached undetectable values after radical prostatectomy [61]. After three decades, PSA continues to be the most widely used oncological biomarker in medicine and is used as a screening method to detect prostate cancer [62]. Although the PSA test has previously been considered the gold standard biomarker for PCa, recent research shows that screening based on PSA does not efficiently

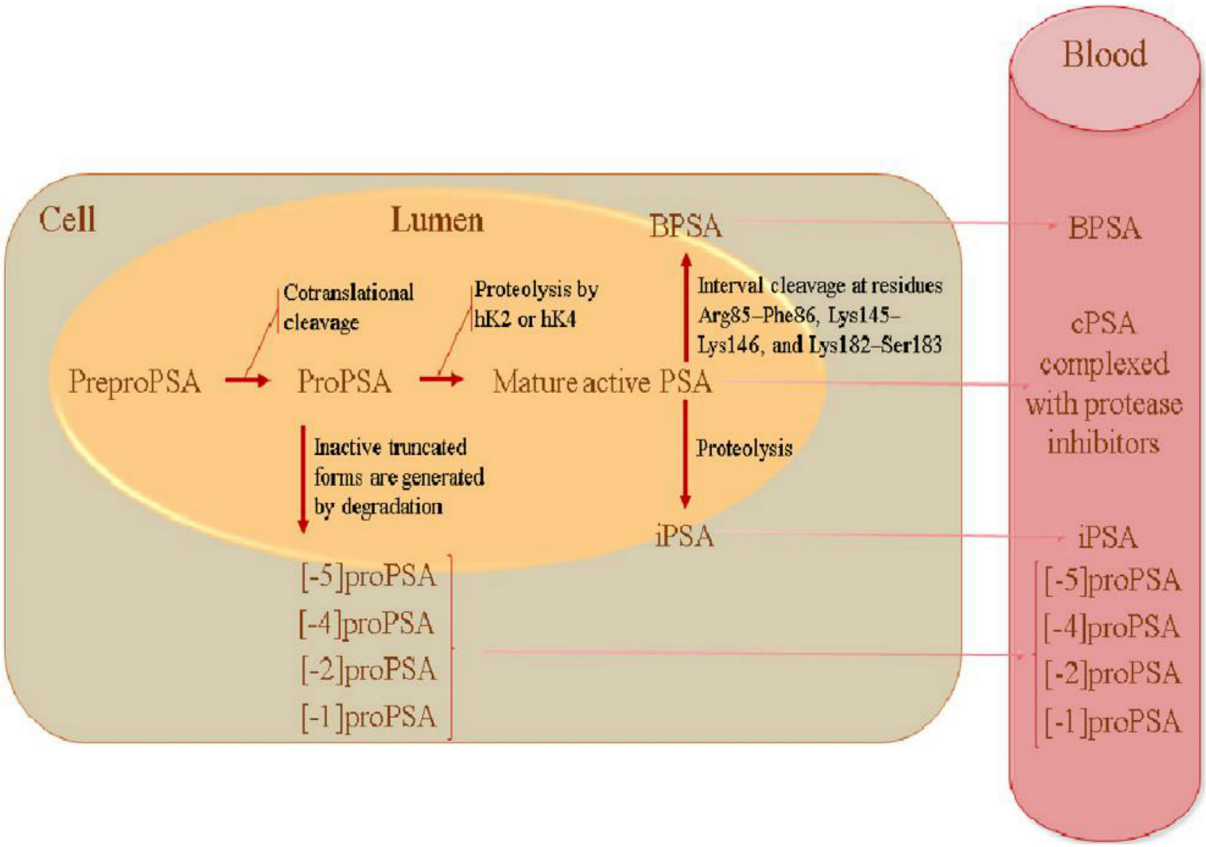

Fig. 4. PSA and biosynthesis of its isoforms. PSA is synthesized as a prepropeptide with 261 amino acids, which is cleaved by cotranslation in the cell to generate [−7]proPSA, an inactive precursor protein. Cleavage within the propeptide yields inactive, truncated variants of PSA. In the lumen, hK2 acts as the major activating enzyme, cleaving [−7]proPSA into mature, active PSA with 237 amino acids. Additional isoforms are cleaved at specific sites, generating BPSA and iPSA. The majority of the mature, active PSA that enters into circulation rapidly binds to protease inhibitors, primarily α1-antichymotrypsin. The remaining, unbound PSA circulates as free PSA.

detect PCa. In view of this, the serum PSA test is complemented with parameters such as PSA isoform-specific tests, PSA kinetics, and PSA density. It is important to note that the specificity and sensitivity of PSA as a diagnostic tool have improved markedly with an increased understanding of the molecular isoforms of PSA [8].

Several studies have illustrated the cancer specificity of proPSA and highlighted [−2]proPSA and [−4]proPSA as important isoforms for the detection of prostate cancer [63–65]. The distinct biochemical properties of [−2]proPSA and [−4]proPSA result in enhanced stability in cancerous tissues, as these isoforms cannot be converted by hK2 in mature PSA. So, increased [−2]proPSA expression could be directly linked with more aggressive cancers [8].

In essence, PSA is an organ-specific biomarker of the prostate, but it cannot be considered a cancer-specific biomarker. Thus, many groups of researchers are proposing the association of glycans with PSA and its tumor specificity for clinical diagnosis.

4. Glycosylation

Glycosylation is an enzymatic process in which carbohydrate molecules known as glycans become attached to other biological molecules such as proteins and lipids, thus affecting different biological pathways. These processes require glycans for a range of purposes, including cell adhesion, endocytosis processes, molecular trafficking and clearance, receptor activation, and signal transduction [66]. Regarding glycans and proteins, the produced glycoproteins are often disposed on cell surface or secreted into the bloodstream. This mechanism is part of the secondary processing of proteins in cells, displaying a critical role in determining protein structure, function, stability, and protection against proteases [67,68].

It is conservatively estimated that around 2% of all genes in the human genome encode for proteins involved in various aspects of glycosylation and that half of all cellular proteins are directly glycosylated in some form [69,70]. Another estimate reports the human proteome as 70% glycosylated [71]. Glycans are attached to proteins via amino groups (*N*-glycans) or hydroxyl groups (*O*-glycans); fucose, galactose, glucose, mannose, *N*-acetylglucosamine, *N*-acetylgalactosamine, and sialic acid are the major building blocks of human *N*- and *O*-glycans [67,72] (Fig. 6).

The structural complexity of glycoproteins arises from several key factors, including various combinations in which monosaccharide subunits can be linked together. Factors such as the anomeric position of the linkage (α or β configuration), the presence and degree of branching, the presence of more than one glycosylation site on each individual protein, and modifications made by non-carbohydrate substituents (such as sulphate, phosphate, and acetate) can be considered [8,73].

In the glycosylation process, a series of glycoenzymes, such as glycotransferases and glycosidases, act upon a protein as it passes through the endoplasmic reticulum to the Golgi apparatus. The structural modifications made by these glycoenzymes affect cell-specific glycan expression patterns. Glycans have numerous functions and can be involved in the correct conformation and folding of the protein. They can affect the above- mentioned characteristics (structure, function, stability, and protection against proteases), in addition to serving as recognition motifs for lectins (carbohydrate-binding proteins) [8].

Briefly, in *N*-glycosylation pre-assembled blocks of sugars are transferred during the amide group transduction of an asparagine

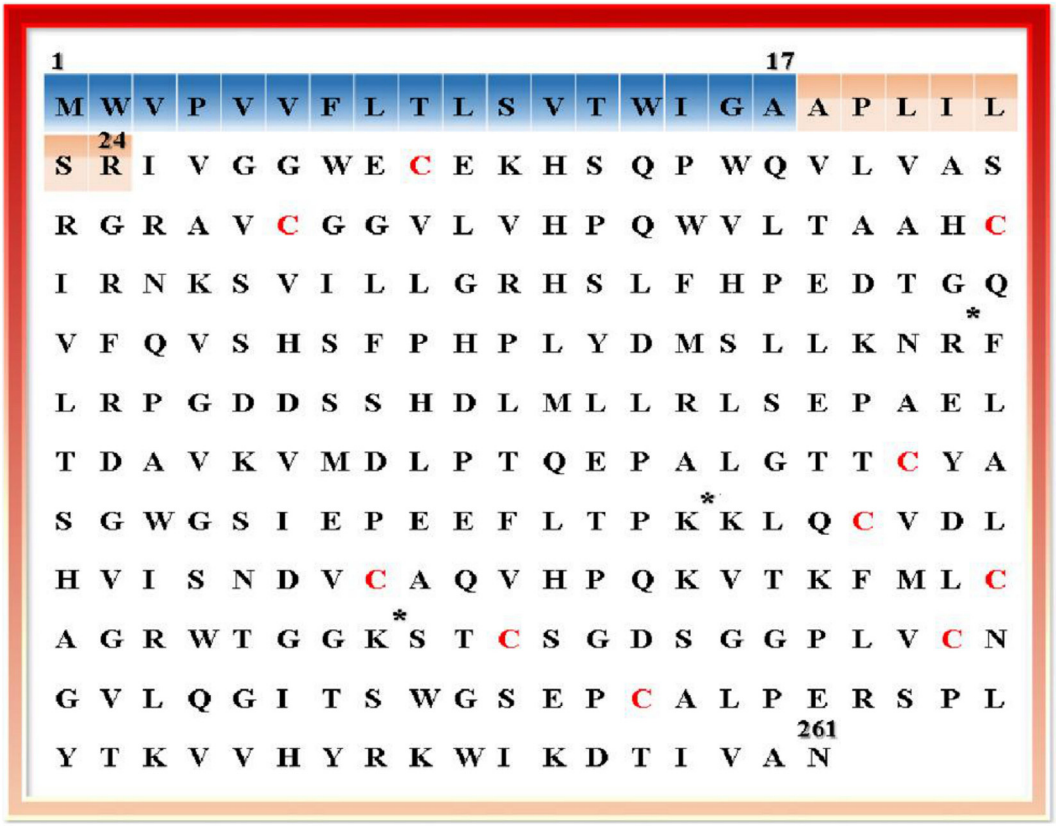

Fig. 5. Complete amino acid composition of PSA with highlighted cleavage points. The 17-amino acid leader sequence is highlighted in blue and the inactive, truncated cleavage region is highlighted in beige. Intrachain cysteine bonds are shown in red, and star symbols denote benign PSA internal cleavage sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

residue, whereas in *O*-glycosylation the sugars are added to the hydroxyl of the amino acids serine and threonine. > 200 types of transferases and other additional regulatory proteins, expressed in specific patterns and levels in each tissue, participate in the synthesis of a diverse set of glycans [74,75].

O-glycosylation is a covalent post-translational modification in which monosaccharides are transferred to amino acid residues of serine (Ser) and/or threonine (Thr) by an *O*-glycosidic bond. The synthesis of *O*-glycans occurs in the Golgi apparatus and is mostly started by the activity of polypeptide *N*-acetylgalactosamine-transferases that link a single *N*-acetylgalactosamine residue to Ser or Thr, thus forming the antigen. In contrast to this glycan type, which is termed mucin-type *O*-glycans, a wide range of non- mucin *O*-glycans exist, namely α-linked *O*-fucose, β-linked *O*-xylose, α-linked *O*-mannose, β-linked *O*-*N*-acetylglucosamine, α- or β-linked *O*-galactose, and α- or β-linked *O*-glucose glycans [76].

In contrast to *O*-glycosylation, *N*-glycosylation occurs during translation of target proteins by the addition of glycan structures to the amino group of asparagine (Asn) residues at the consensus motif asparagine-X-serine/threonine, in which X is any amino acid except proline. Instead of step-by-step addition of single sugar residues, *N*-glycosylation starts with synthesis of a dolichol-bound oligosaccharide precursor in the endoplasmic reticulum, consisting of 14 sugar moieties including mannose 9 residues. Then, this oligosaccharide is transferred to an Asn residue within the nascent polypeptide by the oligosaccharyltransferase protein complex. After the transference, the correct folding and secretion of the glycoprotein depends on trimming of the glycan precursor in the Golgi, resulting in high mannose glycans. Removal of part of the mannose 9 residues by Golgi mannosidases is the prerequisite of formation of complex or hybrid di-, tri- or tetra-antennary glycans. These can then undergo a variety of extensive modifications including sialylation, fucosylation, addition of galactose, *N*-acetylglucosamine, among others, resulting in highly complex and heterogeneous structures [76].

Glycome can be conceived as the set of synthesized glycans by a cell under specific conditions. However, the constitution of a glycome can change dramatically in response to subtle changes in the cellular environment, including extensive changes in glycosylation patterns that characterize many diseases [77].

4.1. Glycosylation in prostate cancer

In cancer, glycans are implicated in a range of purposes, including cell dissociation and cell invasion, cell-to-cell interaction, and cell signaling through cell-matrix interaction [78]. Glycans have been demonstrated to play a role in endothelial cells by ensuring the survival of endothelial cells, regulating vascular permeability, and influencing the connection of blood and lymphatic vessels. In addition, glycosylation is also implicated in immune modulation and cancer metastasis [6,66].

The elucidation of the molecular isoforms of PSA, as it concerns PCa, has enhanced the utility of PSA testing for PCa detection. However, still no single test exists that can distinguish between significant and insignificant disease. Recent advances in the field of glycobiology have shown that aberrant glycosylation patterns are a fundamental characteristic of tumorigenesis, including reduction or increase in expression of a particular structure or pattern, suggesting that modified glycoproteins with tumor-specific glycan moieties are viable targets for cancer detection and diagnosis [8,79].

Specific targeting of the single site of PSA glycosylation for structural characterization of the constituent *N*-glycans as potential disease and stage-specific tumor biomarkers has been a longstanding goal. Multiple studies have the aim to distinguish PCa and BPH. For example,

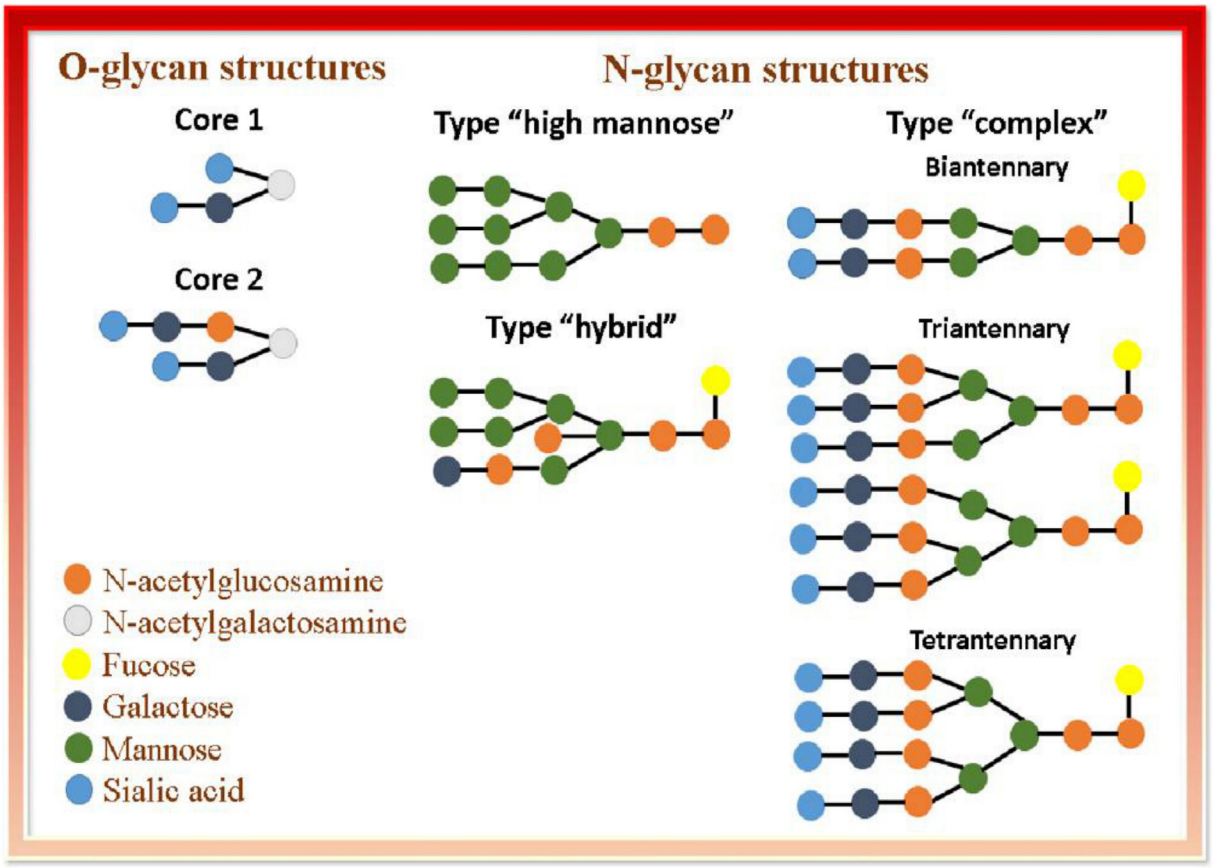

Fig. 6. Typical structures of *N*- and *O*-glycans expressed in glycoproteins in human serum.

the possibility has been investigated of distinguishing different stages of PCa and BPH by analyzing the glycosylation patterns in sera from men with BPH and with PCa in Gleason scores 5 and 7. Also, greater expression of fucosylated and biantennary glycans linked to α2,3 sialic acid has been studied in PCa-derived sera compared to BPH. In addition, a remarkable reduction has been observed in the expression of trigalactosylated and tetrasialylated tri-antennary glycans with a fucosylated outer arm, and increased levels of tetrasialylated tetra-antennary glycans at PCa score 7 in comparison with score 5 [80].

A study based on glycoprotein analysis of tissues from PCa identified 350 glycopeptides, in this case 17 were altered in aggressive PCa; some glycoproteins were also altered, with increased expression (periostin) and reduction (monoamine oxidase) in aggressive PCa [81]. It is possible to observe that alterations in glycosylation are suggestive of cancer and enable the identification of different stages of PCa, which would not be possible through the PSA test.

Changes in the glycosylation pattern of different forms of PSA from PCa and BPH have also been described, most of them related to sialic acid levels. The presence of α2,3 sialic acid residues linked to PSA may be useful to discriminate between malignant and benign diseases when comparing the oligosaccharide profiles of fPSA and cPSA in serum and PCa seminal plasma [82]. Another study characterizing glycans present in isoforms (F1-F5) obtained by two-dimensional PSA electrophoresis revealed differences in the degree of sialylation for F3 and F4 isoforms that can distinguish CaP from BPH. F3, which has mono- and disialylated *N*-glycans, is reduced in PCa when compared to BPH, and decreases gradually with the stage of cancer, while F4, which gradually increases with the PCa stage, presents only the monosialylated form. The results indicated a reduction in sialic acid levels in PCa; the quantification of F3 can aid in the diagnosis of the disease [83].

Another study investigated and compared the levels of fucose and sialic acid in PSA-linked *N*-glycans in sera of patients with BPH and PCa having different degrees of aggressiveness. They found a significant reduction in fucose expression and an increase in α2,3 sialic acid in the PSA of the more aggressive PCa cases; the results allowed authors to distinguish BPH and PCa considered of low risk [7].

These investigations show that the expression of differentiated glycan patterns in both PSA and other glycoproteins may be useful markers for PCa and could offer better specificity than the current serum PSA test.

5. Lectins as biotechnological tools

Lectins are naturally occurring carbohydrate-binding proteins which are able to discriminate different glycan structures and, thus, are useful tools for glycoanalysis. They are involved in crucial physiological events of protein-carbohydrate interactions, such as cell adhesion, migration and interaction [84,85]. Although initially found in plants, lectins are widely distributed among viruses, microorganisms and animals [86]. Unlike antibodies, which are structurally similar, lectins are differentiated by amino acid composition, metal requirement, molecular weight and three-dimensional structure [87].

The association constant of lectins with monosaccharides ranges from 10^3 to $5 \times 10^4\ M^{-1}$, while among lectins with oligosaccharides, 10^4 to $10^7\ M^{-1}$, values included in the same range found for antibody-antigen and enzyme-substrate [88,89] associations. Each lectin binds to a specific monosaccharide, oligosaccharide or glycoconjugate through its binding sites that tend to be located on the surface of the protein. This interaction occurs by hydrogen bonds, electrostatic, hydrophobic and Van der Waals interactions [87,89,90]. However, most of these interactions are made by hydrogen bonds due to the availability of hydroxyl groups in the sugars, allowing for the formation of these bonds among amino acids of the binding site in the lectin and carbohydrate. In addition, water molecules participate as bridges in the formation of

hydrogen bonds between the protein and its ligand.

The great variety of lectins and their potential to recognize different carbohydrates have stimulated the use of these proteins as recognition tools in many biotechnological studies [91]. Since there is specificity for mono- and oligosaccharides expressed on the cell surface, lectins can be used for typing blood cells [92] and also, as insecticides, as they impair the development and cause mortality of larvae [93]. Some of these proteins present antimicrobial action, for example, acting as antibacterial and antifungal agents [94] and as antivirals by preventing viral invasion and replication [95]. They have also been used in affinity matrices for recognition and purification of glycoproteins [96] and in the development of drug delivery systems in mucous membranes [97].

Lectins have been widely used in glycoanalysis to identify changes in glycan composition and to elucidate the physiological and pathological mechanisms related to these alterations, as well as the modifications in regulatory genes of enzymes that participate in the glycosylation process [7,98]. An increase or reduction in branching of glycans and expression of monosaccharides such as sialic acid, fucose and mannose at different stages of tumor transformation, or even the appearance of cancer-associated glycosylated antigens, such as the sialyl-Tn antigen (Neu5Acα2-6GalNAc-O– Ser/Thr), are changes commonly related to cancer progression and metastasis [7,99–101]. The specificity by which lectins recognize the glycans allows for the identification of tumor-specific alterations, leading to the discovery of new glycosylated biomarkers.

Thus, lectins have been reported as potential recognition tools for disease-related glycans, such as those affecting the prostate. For example, the glycosylation profile of PSA in seminal fluid can be studied using three lectins with different specificity: SNA (*Sambucus nigra* agglutinin type I) with specificity for α-(2,6)-terminal sialic acid, LTA (*Lotus tetragonolobus* agglutinin) which binds to α-L-fucose and MAA (*Maackia amurensis* agglutinin II) recognizing α-(2,3)-terminal sialic acid. After incubating the previously mentioned lectins in the immunosensor with attached PSA, a high electrochemical impedance spectroscopy (EIS) response was observed with LTA and SNA when compared to MAA, suggesting higher concentrations of the binding sugars of LTA and SNA lectins in PSA glycan [102]. In another study, α-2,3 sialic acid linkage was found only on PSA of cancerous origin; this finding was made using MAA lectin in an immunoassay [103]. The glycoprotein fetuin has been applied as an analyte for a biosensor constructed with Cramoll, a glucose- and mannose-specific lectin, extracted from seeds of a leguminous plant, *Cratylia mollis*. Cramoll, when immobilized on a nanostructured electrode, has been shown to be able to differentiate prostate cancer and benign prostatic hyperplasia [104]. Therefore, it is necessary to search for new lectins that may detect aberrant glycans expressed in pathology that affect the prostate; this class of proteins is ubiquitous in nature and can be obtained by easy techniques, in addition to being able to detect subtle changes that may help diagnosis.

6. Conclusion

PSA is an *N*-glycosylated protein that has been used for diagnosis and monitoring of PCa. However, PSA levels are unable to distinguish aggressive from non-aggressive PCa and other benign prostatic diseases. Altered glycosylation may be expressed in abnormal cells and accompany many diseases, including tumor progression. These changes can be identified as potential glycan biomarkers by comparing the glycosylation profiles between tumor and normal tissues or biofluids at early disease stages, which can potentially improve diagnosis, treatment and patient survival. The identification of potential new biomarkers has been facilitated by glycan-based techniques. Glycans and glycoproteins show specific indications for PCa diagnosis, aggressiveness and prognosis. PSA and other glycoproteins produced in PCa express aberrant glycosylation, which could be used to distinguish between healthy and diseased prostate. The ability of lectins to detect different types of glycan biomarkers has encouraged the development of lectin-based strategies for selective capture of these glycotargets for cancer in tissues and biofluids. However, confirmatory studies from technical to biological perspectives are required for development of clinically diagnostic, quantitative assays based on glycan biomarker detection.

Declaration of Competing Interest

The authors declare that no competing interests exist.

Acknowledgements

This manuscript has been elaborated in collaboration among all authors. PMSS, WFO and PBSA performed the bibliographic survey and wrote the manuscript. MTSC and LCBBC designed, supervised and corrected the study. The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) is acknowledged for fellowships (LCBBC and MTSC) and grants. Moreover, authors are also grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and to the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

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