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REVIEW ARTICLE

Predictive Perspectives of Disease—Transformed Protein Biomarkers

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ABSTRACT

With advancement in instrumentation, computation and understanding of disease etiology, proteomics has been expanded to harness the knowledge of change in protein folding and misfolding, protein-protein interaction, protein modification, etc. during progression of disease which is a source of discovery for various biomarkers including predictive biomarkers. Various methodologies for disease prediction are reported using 'omics' technology; however, advancement in proteomics with discovery of protein biomarker allows for the estimation of disease risk from years to decades before any disease even manifests internally. Specific proteins as disease biomarkers that appear in the body fluid/diseased tissues are generally measured. Recently, new proteomics technologies are also being developed in order to facilitate both the high-throughput and high-sensitivity requirements of disease-related applications of proteomics and possibly providing the framework for prediction of diseases. Therefore, there is a growing interest in proteomics technologies to discover processes that are involved in various diseases, to discover new biomarkers that correlates with the prediction and early detection of diseases. Now there is change in research thinking where already known biomarkers alone or in combination of others are under investigation for advanced application like in prediction and early detection of chronic diseases. In this review, we have emphasized the prediction perspective of some of the protein biomarkers like CA-125, Lp-PLA_2 and tau protein for diseases like cancers, cardiovascular diseases, and Alzheimer's respectively.

Keywords: CA-125, Lp-PLA_2, Predictive biomarker, Protein biomarker, Tau protein.

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INTRODUCTION

Presently, global disease burden is showing worrying trend which is a major concern of disease management. Unfortunately, the burden of chronic diseases is rapidly increasing worldwide. The burden of the proportion of noncommunicable diseases (NCDs) is expected to increase to 57% by 2020.[1] Recent projection on chronic diseases indicated that, by 2020 these diseases will account for almost three-quarters of all deaths worldwide. Projections on communicable diseases also indicate that these would also occupy critically important positions up to 2020[2] and 82% of 'premature' deaths would occur in low and middle income countries in the age group of 70 years and above. Certain diseases like cardiovascular diseases, malignancies, chronic respiratory diseases, neurological and mental disorders as well as musculoskeletal diseases shall be on the rise in those aged 60 years and above.[3,4]

Disease detection techniques has played very crucial role in disease management. Various techniques based on colorimetric, fluorescence and radiometric methods gave a baseline platform for disease detection, whereas enzyme-linked immunosorbent-assay (ELISA), Biorobotic, Biochip, Biosensor, Imaging techniques, microscopy and more recently nanotechnology have supported as add on for the disease detection and has revolutionized the detection methodology in favor of effective disease management. Recently, interdisciplinary scientific intervention and the basic understanding of etiology of diseases have been tried for identification of several disease specific biomarkers for better disease management. However, rationalization of detection techniques at the level of early detection and prediction is still a major challenge.

Proteins, which are the principle constituents of the protoplasm of all cells, undergo transformations under different conditions of stress. The study of all the proteins expressed by the genome under stressful conditions of ageing and others, provide insights into the health of the genome. In a herd of multiple thriving cells and tissues, the conditions of change/transformation get embedded and expressed within the dynamic change of nucleotide sequences of the cell population, the

surrounding ribonucleic acids (RNAs) and eventually within the transformations of the proteins expressed by the integrated genome pool. Not all the cells in the herd get transformed into diseased cells at a time. However, the gradual transformation of the healthy cells into the diseased ones leave several 'transformed metabolites' which can be identified over a period of time by use of sophisticated instruments and measurements. Great insight can be had from such studies about the health conditions of the tissues by studying such 'transformed metabolites' and especially the protein pools thus expressed.

Advancement in proteomics research and technology helps for discovery of various protein biomarkers including predictive biomarker. Besides traditional ways of identification and characterization of proteins, several new techniques like matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)[5,6] and surface-assisted laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS)[7] have contributed significantly in protein profiling of various samples, and thereby the disease detecting technology. The techniques of protein-chip arrays have also been used to understand protein-protein interactions for a wide range of diseases, this made base platform for discovery of biomarkers.[8,9] The advancement in proteomics technologies have led into high-throughput and high-sensitivity assays. Nanotechnology has also offered some unique diagnostic capabilities for development of highly selective and sensitive assays[10] in favor of discovery of certain biomarkers. This advancement in proteomics opens up an innovative platform for discovery of various protein biomarkers which will allows for the identification and prediction of various diseases on the basis of protein structure and function. Using such studies many diseases can be predicted well in advance.

Research in biomedical instrumentation is also going in high pace for development of advanced version of instruments which can fulfill the requirement of very high detection efficiency and efficacy. Advancement in these instrumentations led to its application in discovery of various biomarkers and this is playing important role in effective disease management. It helps in prediction of disease, early diagnosis, disease prevention, drug target identification, drug response, etc. These biomarkers can be discovered using various OMICS platforms, such as genomics,[11] proteomics,[12,13] lipidomics,[14,15] etc.

Genomics platform using techniques, such as Northern blot, Gene expression, polyacrylamide gel electrophoresis (PAGE), deoxyribonucleic acid microarray (DNA microarray) etc. have played important role in discovery of biomarker, whereas in proteomics approach with advancement in instrumentation has opened fine gateway for discovery of biomarkers to diagnose disease. Secretomics, a subfield of proteomics has recently emerged as an important tool for the discovery of biomarkers[13] that is related to secretion of proteins and secretion pathways using proteomic approaches. Multiple modern techniques like MALDI-TOF-MS and SELDI-TOF-MS, Ab microarray have been explored for discovery of biomarkers which is boosting the disease detecting technology for protein profiling in various samples. Recently, matrix-assisted laser desorption/ionization (MALDI) have been employed widely for rapid determination of proteins in particular mixtures for proteomic study. Fluorescence two-dimensional differential gel electrophoresis (2-D DIGE) can be used to quantify variation in the 2-D DIGE process and establish statistically valid thresholds for assigning quantitative changes between samples.[16] The techniques of protein-chip arrays have been extensively used to understand protein-protein interactions for a wide range of diseases. Table 1 summarizes the main advanced techniques in proteomics that are used for the discovery of biomarkers for disease detection.

Computational predictive models are also boosting the discovery of various protein biomarkers.[17] Application of this have been demonstrated in which extensive and diverse feto-maternal protein trafficking occurs during pregnancy can be readily detected non-invasively in maternal whole blood.[18] The proteomic networks contain many biomarkers that are proxies for development. It extended into potential clinical application which can be used to monitor normal and abnormal fetal development.

Recently, nanobiotechnology application has been extended to improve the discovery of biomarkers. One such example being the sensitive detection of multiple protein biomarkers by nanobiosensors for the management of cancer made most important chapter of nanomedicine. Nanobiotechnology has refined and significantly implemented to molecular diagnosis of cancer through the use of gold nanoparticles and quantum dots. Therefore, it is an expectation and hope nanobiotechnology will facilitate the combination of

Table 1: Advanced techniques in proteomics for discovery of biomarkers

Techniques
• MALDI-TOF-MS
• SELDI-TOF-MS
• Protein-chip array
• Ab microarray
• 2D-PAGE
• Fluorescence two-dimensional differential gel electrophoresis (2D DIGE)
• Nanobiotechnology
• Computational predictive models

diagnostics with therapeutics which is an important feature of a personalized medicine approach to cancer. For diagnosis and targeted delivery of cancer therapy monoclonal antibody nanoparticle complexes are under extensive investigation.

Disease Prediction and Protein Biomarker

In coming year, disease detection aspect is going to be key factor in disease management. Although more basic research may be needed on some important aspects of the mechanisms of disease progression, biomarker of disease detection is going to be the focal point for future disease detection. However, rationalization of disease detection at the level of prediction is major challenges of disease detection. The future of medicine's focus may potentially shift for preventing disease rather than treating existing diseases, typically late in their progression. Therefore, a new philosophy in healthcare has emerged which will provide platform for personalized patient's treatment. Predictive diagnostics is considered as the basis for targeted preventive measures and consequent development of individualized treatment approaches. One of the best examples in this category is a paradigm shift toward personalized cancer medicine.[19] Therefore, there are several opportunities for discovery for new predictive biomarkers for predictive diagnostics which will strengthen the platform for joint venture for R&D, regulatory and increased market share of diagnostics. Now, there are opportunities in this direction for new global and national actions which includes strengthened interaction and partnerships, regulatory, legislative and fiscal approaches, etc.

There are various prediction methodologies which include genomics, proteomics, cytomics, etc. but the most commonly used method for prediction of disease is based on genetics. However, the presence of faulty genes of diseases does not necessarily mean that someone will get the disease.[20] It is considered that lifestyle and environment has significantly played role in development of common and complex diseases in the wider population but is not affected only by heredity. Therefore, genes are not perfect predictors of future disease development and progression. However, advancement in proteomics with discovery of predictive biomarker allows for the estimation of disease risks years to decades before any disease even manifests internally which can offer lifestyle advice or medication with the aim of preventing/delaying the predicted illness. A protein being the predictive biomarker has a number of advantages over others. Determining the body fluid's secretary proteins can be important for protein function annotation and disease biomarker discovery.[21] This approach indicates that the network-based prediction method is quite promising. It is anticipated that the method will benefit the relevant areas for both basic research and applied research. Knowledge on systematic configuration of protein structure may also help in discovery of biomarker and it may act as predictor of particular disease. In this direction three-dimensional (3D) structure elucidation of proteins may help improving the prediction of disease-related variants.[22] Protein interaction is a well-known phenomenon in biological system; therefore, protein interaction background could provide important clues to help better illustrate single aminoacid polymorphisms (SAPs) functional association which is responsible for the majority of human-heritated diseases. This research will facilitate the post genome-wide association studies. Network features are found to be most important for accurate prediction and can significantly improve the prediction performance.[23] Protein folding and misfolding have played important role in disease development and progression; therefore, harnessing the knowledge of misfolding and folding of protein during disease progression can be channelized into disease prediction. A number of diseases are mediated by mutation-induced protein misfolding.[24] Unfolded protein to misfolding disease is classical example of this category.[25] It is found that an unstructured protein possess destructive potential during progression of disease.[26] Many proteins also undergo wide variety of chemical modifications after translation. The post-translational modifications are very critical to the protein's function which is also recognized for its important role in disease development and progression.[27] One such classical example of modification of protein is phosphorylation which occurs to many enzymes and structural proteins in the process of cell signaling. In addition to phosphorylation, proteins can be subjected to other modification, such as ubiquitination, methylation, acetylation, glycosylation, oxidation and nitrosylation, which all can contribute to diseases. Therefore, degree of protein modification and their pattern can be explored as predictor of disease progression and development. Although in many diseases enzyme is considered as biomarker still there is need to understand the kinetics pattern of respective enzyme during disease progression which may help for discovery of predictive biomarker for disease prediction.

Prediction Perspective of CA-125 Biomarker in Cancer

There are enormous changes in diagnosis of cancer from turn-around-time to automation has become a major catalyst in this growth. Tremendous support of molecular techniques like fluorescent *in situ* hybridization (FISH),

polymerase chain reaction (PCR) and microarrays are recorded in cancer diagnosis. In recent years, biomarkers have gained importance by oncologists. The mix and match of molecular and protein markers has given platform for the whole new generation of tests. The integration of advanced proteomics with informatics for disease-related expression profiles can be used for identifying high-risk groups with much more reliability and it will allow us monitoring preventive strategies. In recent year, several proteins have been considered as biomarkers for early detection of certain types of cancer, still there is need of search for more predictive protein biomarker for various other types of cancers. During past decades several tumor markers have been enlisted that enable diagnosis, monitoring, and screening. Some biomarkers have found their way to clinical use. The most notable ones are alpha-fetoprotein (AFP) which appears in primary liver cancer and some rare forms of testicular cancer. Carcinoembryonic antigen (CEA) is another important biomarker which can help detect colorectal cancer. Cancer antigen 125, carcinoma antigen 125, or carbohydrate antigen 125 (CA-125) is also known as mucin 16. It is a well-established biomarker for ovarian cancer. Prostate-specific antigen (PSA) appears in prostate cancers. There is hope that advanced proteomics together with accelerated informatics platform can provide disease-related expression profiles in specific combination that could identify high-risk groups with much more reliability and will allow developing preventive strategies.

Now there is change in research thinking where already known cancer biomarker alone or in combination with others is under investigation for advanced application like its prediction and early detection of disease. Recently researchers found out that CA-125 protein may help detect ovarian cancer in its early stage.[28] Cancer antigen 125 is an important marker in cancer detection.[29] Schematic diagram of the structure of CA-125 is provided in Figure 1.

OVA1 test^{TM}—the first blood test cleared by the US food and drug administration (FDA) can indicate the probability of cancer in an ovarian mass disease. Ovarian cancer is a silent killer as its symptoms are very nondescript. Under normal circumstances, a physician is unable to know the ovarian mass is either caused by cancer or something else until operates and tests it. The test utilizes five well-established biomarkers in well define combinations and proprietary software to determine the likelihood of malignancy in women with ovarian mass for whom surgery is planned. This includes established blood tests known as tumor markers: Cancer antigen 125 (CA-125 II), Transthyretin (TT or prealbumin), apolipoprotein A-1 (Apo A-1), Beta2-microglobulin (Beta2M), and Transferrin (Tfr). The results of these blood tests are combined in an equation to produce a single numerical score. It is also found that elevated CA-125 is sufficient to detect 80 to 90% of recurrences of ovarian cancer.[30,31] It is surprising to know that menopausal females with an elevated CA-125 and without ovarian cancer are exposed to an increased risk of premature mortality.[32] Investigations tells us that high CA-125 levels (>150 U/ml) can rule in the presence of atrial fibrillation in patients with heart failure.[33] Recently, potential of serum CA-125 along with L-amino acid oxidase (LAAO) also highlighted in future predictor of cancer recurrence.[34]

There are several established markers of cancer, which have been validated over the years. Important among such biomarkers are tabulated in Table 2.

Prediction Perspective of Lp-PLA$_2$ in Cardiovascular Diseases

Several diagnostic tests have been used for detection and progression of heart disease including protein biomarkers. Of the lipid variables, the ratio of total cholesterol to high density lipoprotein (HDL) cholesterol and Apo lipoprotein B-100 were the most powerful predictors. In cardiovascular disease atherosclerosis is now recognized to be an inflammatory process. Four

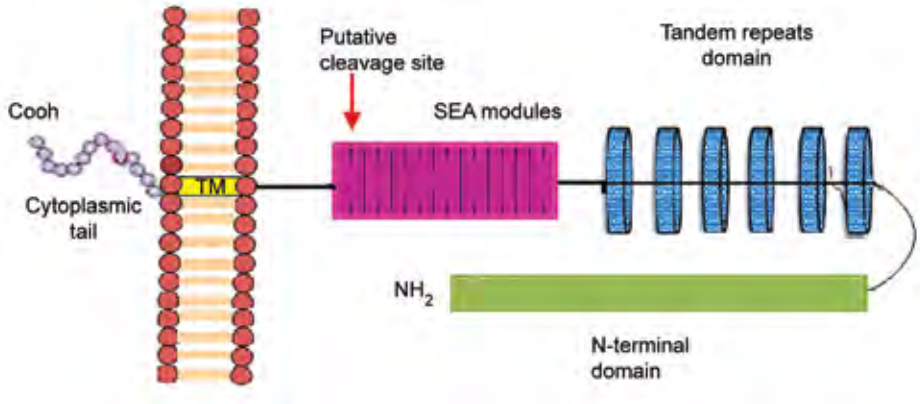

Fig. 1: Structure of CA-125 (Mucin 16) Adapted and derived from Wikipedia https://en.wikipedia.org/wiki/CA-125

Table 2: Some important biomarkers in cancer detection

Biomarkers
• Alpha-fetoprotein (AFP) of liver cancer and rare form of testicular cancer
• Carcino-embryonic antigen (CEA) in colorectal cancer
• CA-125 for ovarian cancer
• Prostate-specific antigen (PSA) in prostate cancer
Matrix metalloproteinases (MMPs) and
A disintegrin and metalloproteinases (ADAMs)

markers of inflammation were found to be significant predictors of the risk of future heart attacks—C-reactive protein (CRP) test, Serum amyloid A, interleukin 6, and soluble intercellular adhesion molecule-I (sICAM-I). However, C-reactive protein has been identified as one of the most significant risk factors for cardiovascular disease and heart attacks. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is also considered as risk factor for the development of atherosclerosis.[35] Lipoprotein-associated phospholipase A_2 is highly specific biomarker for vascular inflammation linked with increasing cardiovascular (CV) risk level. It is considered to be an independent risk marker, as well as an additive to the predictive value of high-sensitivity C-reactive protein in assessing myocardial infarction (MI) risk in moderate-risk populations. Increased Lp-PLA_2 level is independently associated with coronary artery disease (CAD) severity, and Lp-PLA_2 level may be used to discriminate those who are at increased risk of cardiovascular disease.[36]

A Lp-PLA_2 also known as platelet-activating factor acetylhydrolase (PAF-AH) is an enzyme that in humans is encoded by the phospholipase A2, group Homo sapiens VII (PLA2G7) gene. Lipoprotein-associated phospholipase A_2 is a 45-kilo Dalton (KDa) protein of 441 amino acids. It cleaves oxidized fatty acids from lipids in plasma mainly carried by low-density lipoprotein cholesterol (LDL-C). Elevated levels of Lp-PLA_2 (>200 ng/dl) predict a 40 to 400% (avg~100%) increased risk for MI and stroke (adjusted for cardiovascular disease risk factors). Further, a growing number of preclinical and genetic studies support a causal role of Lp-PLA_2 in atherosclerosis. The development of a novel therapeutic agent that directly inhibits the Lp-PLA_2 enzyme has provided a unique opportunity to directly test the hypothesis that inhibition of this inflammatory enzyme will translate into improved clinical outcomes.[37]

The vast majority of plasma Lp-PLA_2 mass binds to low-density lipoprotein (LDL) while a smaller amount is associated with high-density lipoprotein (HDL). Lipoprotein-associated phospholipase A_2 is also bound to lipoprotein (a) [Lp(a)], very low-density lipoprotein (VLDL) and remnant lipoproteins. Several lines of evidence suggest that the role of plasma Lp-PLA_2 in atherosclerosis may depend on the type of lipoprotein particle with which this enzyme is associated. Data from large caucasian population studies have supported plasma Lp-PLA_2 (primarily LDL-associated Lp-PLA_2) as a cardiovascular risk marker independent of, and additive to, traditional risk factors. On the contrary, the HDL-associated Lp-PLA_2 may express antiatherogenic activities and is also independently associated with lower risk for cardiac death.[38] A schematic diagram showing the role of Lp-PLA_2 in developing atherosclerosis is provided at Figure 2.

The PLAC™ test for Lp-PLA_2 is launched which is the blood test that helps identify hidden risk for heart attack and stroke. Early detection of Lp-PLA_2 by this test disease can be prevented by more aggressive treatment. The PLAC® Test for Lp-PLA_2 is a blood test cleared by the FDA to aid in assessing risk for both coronary heart diseases (CHD) and ischemic stroke associated with atherosclerosis.[40]

A number of protein biomarkers for detecting cardiovascular diseases are in Table 3.

Alzheimer's Disease: Prediction Perspective of Amyloid 'tau'

Diagnostic tools and criteria have been developed in recent years to make a clinical diagnosis of Alzheimer's disease (AD) with an accuracy rate of 85 to 90%. The factors used to complete a diagnosis include: medical history, mental status evaluation, physical examination, neurological examination, neuropsychological evaluation, brain scans, laboratory tests, biomarkers etc. Recently

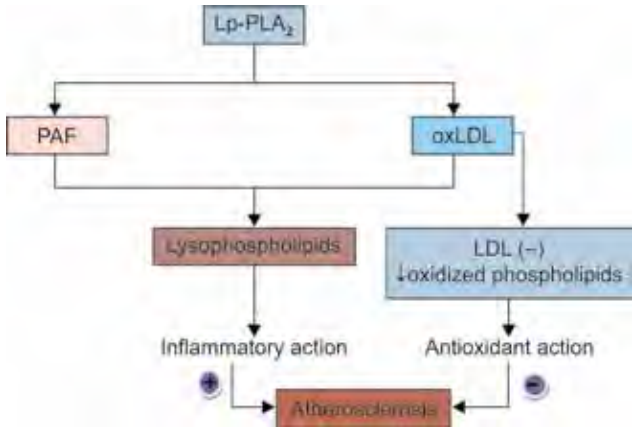

Fig. 2: Schematic diagram of role of Lp-PLA_2 (adapted and derived from reference number 39)

Table 3: Protein biomarkers in cardiovascular disease

Biomarkers
• C-reactive protein
• Serum amyloid A
• Interleukin-6
• sICAM-1
• Surfactant protein-D
• Apolipoprotein B-100
• Lp-PLA_2

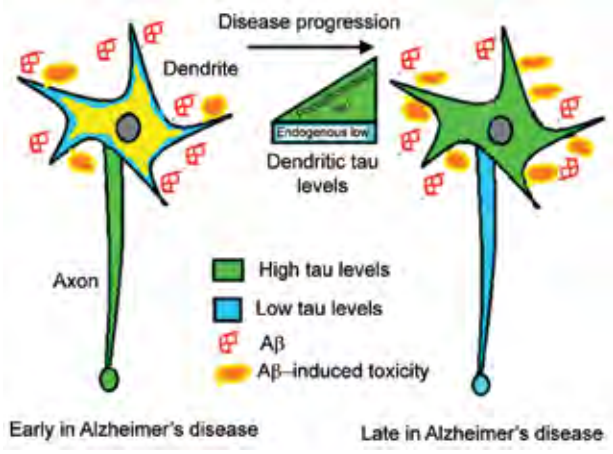

Fig. 3: Change in the content of phosphorilated *tau* after the onset of AD (Adapted and derived from the article by Jim Schnabel, Date 20 May, 2013. The DANA Foundation http://dana.org/News/Details.aspx?id=43549 Date 01/01/2015 time; 17.17 PM

protein based markers have been discovered which can be found few years before the onset of symptoms, creating a new preclinical stage.[41] The biomarkers include amyloids, which are abnormal proteins that accumulate in the brain of a person with AD which causes neuro-degeneration. This predictive biomarker called amyloid 'tau' accumulates inside brain cells, causing the cells to die. Biomarkers flagged in the new diagnostic criteria may be the key to predicting AD before a person exhibits any symptoms. The biomarkers include amyloid accumulation and neurodegeneration. Many scientists believe the great accumulation of amyloids plays a pivotal part in brain damage. In neurodegeneration, the biomarker called 'tau' accumulates inside brain cells, causing the cells to die.[42-44] Recently, it was demonstrated that 'tau' is extensively post-translationally modified by lysine acetylation, which impairs normal tau function and promotes pathological aggregation. The identification of 'tau' as an acetyltransferase provides a framework to further understand 'tau' pathogenesis and highlights 'tau' enzymatic activity as a potential therapeutic target.[45]

It has been suggested that exogenous human P301L 'tau' induces synaptosomal distribution of 'tau' protein with a certain amount of phosphorylation. Regulating the synaptosomal 'tau' level might be a potential target for a therapeutic intervention directed at preventing neurodegeneration.[46] Cerebrospinal fluid (CSF) levels assessment of Aβ1-42 and 'tau' proteins may be accurate diagnostic biomarkers for the differentiation of preclinical AD from age-associated memory impairment, depression and other forms of dementia in patients with mild cognitive impairment (MCI). The recent results confirm the key role of CSF biomarkers in predicting patient conversion from MCI to dementia. The study suggests that CSF biomarkers may also be reliable in a real world clinical setting.[47]

CONCLUSION

Disease detection techniques are perceived by researchers as one of the most important aspect of disease management. There are enormous challenges for scientists to overcome the issues at the level of early detection and prediction of disease. Advancement in proteomics allows for the identification and discovery of various protein biomarkers. Protein-3D structure, Protein misfolding, protein-protein interaction, protein modification and kinetics of catalytic protein can be explored for discovery and design of novel predictive biomarkers. Using such studies many diseases can be predicted well in advance. In this review, we have focused mainly on predictive perspective of protein-based biomarkers in some diseases like certain types of cancer, cardiovascular diseases and AD. These markers are for identification and prediction of these diseases on the basis of presence and functionality of certain identified specialized proteins. The review would provide useful lines and linkages for adopting further research in these areas.

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