INTRODUCTION

Ovarian cancer (OC) represents the most challenging of gynecological malignancies: approximately 70% of OC are diagnosed in an advanced stage where only 20% of patients survive at 5 years, with mortality rate largely unchanged for many years. The high fatality-to-case ratio associated with OC is partially caused by the lack of a recognizable pattern of symptoms in its early stages. This disease has a 5-year survival rate of 90% if diagnosed early (stage I or II carcinoma). Clearly, the development of new methods for early ovarian cancer diagnosis will likely contribute to improved patient outcomes *(Colombo et al., 2006).*

The only well-validated ovarian cancer tumor marker, CA- 125, was discovered about 20 years ago. CA-125 has clinical value for disease monitoring, and it is used as an aid for the early detection of relapse and for assessing response to treatment, but unfortunately Studies have shown that only 50% of women with stage I disease will have elevations in CA 125. On the other hand, elevated levels were absent in 15% of advanced stages *(Collins et al., 2009).*

The sequencing of the human genome has raised hopes that new cancer biomarkers may soon be discovered. By using whole-genome mining approaches, investigators have identified many candidate biomarkers for ovarian cancer diagnosis and prognosis *(Collins et al., 2009).*

In humans, tissue kallikreins (KLKs) are encoded by 15 structurally similar steroid hormone – regulated genes, that co localize to chromosome 19q 13.4, representing the largest cluster of contiguous protease genes in the entire genome. KLK are widely expressed in diverse tissues and implicated in a range of normal physiologic functions from the regulation of blood pressure and electrolyte balance to tissue remodeling, prohormone processing, neural plasticity, and skin desquamation. Data also suggest that KLKs may be causally involved in carcinogenesis, particularly in tumor metastasis and invasion *(Borgono and Diamandis, 2004).*

Many kallikreins have been identified as promising diagnostic and/or prognostic biomarkers for several cancer types, including ovarian, breast, and prostate. Among these kallikreins, prostate specific antigen (PSA) is the best cancer marker. Indeed, many kallikreins seem to be disregulated in ovarian cancer,

and their transcript levels seem to have either favorable or unfavorable prognostic value *(Klokk et al., 2006).*

Several KLK genes are reportedly up-regulated in ovarian cancer as evidenced by numerous studies of altered KLK transcript and KLK protein levels in the tumor tissues, cell lines, ascitic fluid, and/or serum of patients with this malignancy *(Borgono et al., 2004).* It is believed that multiparametric analysis of many different markers offers several advantages over a single biomarker. In this respect, kallikreins, are highly suited for such multiparametric analysis *(Zheng et al., 2007)*.

AIM OF THE STUDY

The aim of the present essay is to study kallikreins as new ovarian cancer biomarkers that are suitable for early disease diagnosis and prognosis which may ultimately lead to improved patient management and outcome.

I. Kallikreins

A. Introduction:

Proteases/Peptidases are defined as enzymes that catalyze peptide bond hydrolysis, and perform fundamental functions in all living organisms. The "degradome" or complete set of proteases expressed at a given time within a cell, tissue, or organism comprises 2% of all genes in many organisms. The human genome contains at least 553 protease genes. Protease action is always irreversible and can involve indiscriminant and non-specific degradation of protein substrates, as in apoptosis, or highly specific proteolytic processing or limited hydrolysis of selected target proteins, resulting in a functional change, as in prohormone activation *(Borgono and Diamandis, 2004).*

Proteases are classified according to location of the scissile peptide bond within the substrate (terminal or internal); as either exo- or endopeptidases, respectively. According to the catalytic mechanism, endopeptidases are divided into the well-known cysteine, serine, threonine, aspartic, and metalloprotease subgroups *(Rawlings et al., 2004).*

Serine proteases were among the first enzymes to be studied extensively, including their structural

characteristics, catalytic mechanism, and roles in normal physiologic processes (e.g., digestion, coagulation, and cellular and humoral immunity) and in the pathology of many diseases (e.g., cancer, neurodegenerative disorders). With the exception of a small class of membrane-bound serine proteases, the vast majority are secreted *(Borgono and Diamandis. 2004).*

Of the 178 human serine proteases, accounting for the 32% of all proteases, the human tissue kallikreins represent the largest contiguous cluster of protease genes in the human genome. *Human* kallikrein 1 (KLK1), human kallikrein 2 (KLK2) and prostate-specific antigen (PSA, KLK3), were the first members of this family to be studied, with KLK1 showing abundant levels in the pancreas (derived from the Greek 'kallikreas'), from which these genes derived their name *(Klokk et al., 2006).*

Between 1994 and 2001, the kallikrein family expanded to include 15 genes and a complete description of the human kallikrein locus was reported. The newly identified kallikreins share significant similarities to the KLK1, KLK2 and PSA kallikreins. These newly discovered kallikreins all map to the same chromosomal region (19q13.4) as the three classical kallikreins *(Yousef et al., 2000).*

The Kallikrein proteins were first described for their kininogenase activity that generates the vasoactive peptides bradykinin (BK) or kallidin (Lys-BK) from kininogen Today, kallikreins have come to denote a group of structurally related serine proteases that are part of the specific gene cluster, mainly due to sequence and structural similarity, and they are not necessarily enzymes with kininogenase activity ***(Klokk et al., 2006).***

B. Classification of Kallikreins:

1) Plasma Kallikreins (Contact system activation of plasma)

Chromosomal localization of the human plasma kallikrein gene was mapped to the q34 – q35 region of the long arm of chromosome 4. Plasma kallikrein, a serine protease, is encoded by a single gene, KLKB1, and synthesised in the liver. It is predominantly secreted by hepatocytes as an inactive molecule called prekallikrein that circulates in plasma as a heterodimer complex bound to HK (high molecular weight kininogen). Prekallikrein is a single chain α-globulin that is present in the plasma of humans and of other animal species at a concentration of 35 – 50 μg/mL. About 80 – 90% of prekallikrein is normally complexed to HK ***(Moreau et al., 2005).***

The plasma kinin forming system, also called the contact system of plasma, consists of 3 serine proenzymes (factor XII or Hageman factor, factor XI, and prekallikrein) and the kinin precursor HK. Contact of plasma with traumatized vessels leads to the binding and auto activation of factor XII (Hageman factor) to factor XIIa, factor XIIa autoactivates factor XII that require the presence of prekallikrein as a cofactor (fig 1). Factor XII activation is the first step in the initiation of the intrinsic clotting cascade *(Ciesla, 2007).*

In vitro, non-physiologic substances, such as glass (negatively charged silicates), carrageenan, kaolin, and a sulfated polysaccharide dextran sulfate activate the contact system of plasma *(Kaplan et al., 2002).* In vivo, the physiologic surface remains unknown. Pathologic initiators may include proteoglycans (sulfate residues on heparin sulfate or chondroitin sulfate or mast-cell heparin). Endotoxins (lipopoly saccharide (LPS)) and crystals of uric acid or pyrophosphate have also been hypothesized to be pathological activators. This intrinsic coagulation/kinin-forming cascade appears to be in equilibrium in plasma even in the absence of any exogenous surface. That is, activation occurs continuously at a finite rate, but is held in check by plasma inhibitors *(Moreau et al., 2005).*

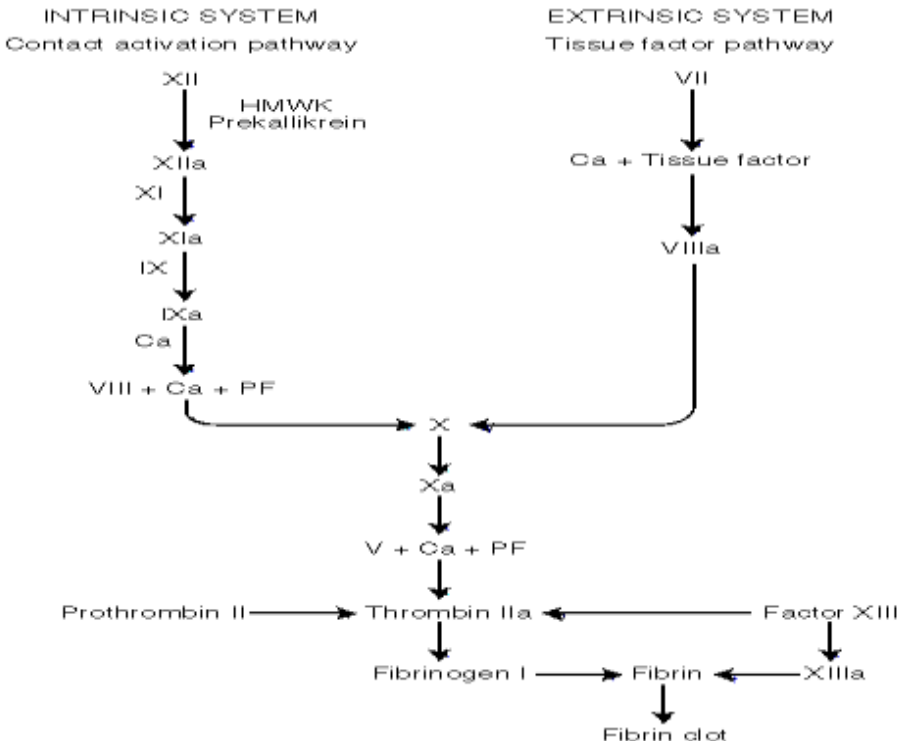

Figure (1): The coagulation cascade *(Ciesla, 2007).*

Factor XII activation is not only a first step in the initiation of the intrinsic clotting cascade and the generation of kinins, but it also leads to the activation of the complement pathway *(Merlini et al., 2004).*

Another mechanism for initiation of the activation of the kallikrein-kinin system depends on binding of components of the contact activation cascade on the surface of cells as leukocytes, platelets, endothelial cells, and myocytes. HK specifically binds to platelets, granulocytes, and endothelial cells in a zinc-dependent, saturable and reversible reaction. This

binding involves both the heavy (domain 3) and light (domain 5) chains of HK, which could be considered as a receptor for prekallikrein on endothelial cells. Binding of HK to endothelial cells leads to activation of prekallikrein to kallikrein and presumably a release of BK (Bradykinin) from HK *(Moreau et al., 2005).*

The fibrinolytic system is responsible for the dissolution of a clot. Fibrin clots are not intended to be permanent. The purpose of the clot is to stop the flow of blood until the damaged vessel can be repaired. Fibrinolysis is the process by which the hydrolytic enzyme plasmin digests fibrin and fibrinogen, resulting in progressively reduced clots. This system is activated in response to the initiation of the activation of the contact factors. Plasmin is capable of digesting either, fibrin or fibrinogen as well as other factors in the cascade (V, VIII, IX, and XI). Normal plasma contains the inactive form of plasmin in a precursor called plasminogen. This precursor remains dormant until it is activated by proteolytic enzymes, the kinases, or plasminogen activators. Reduced fibrinolytic activity may result in increased risk for cardiovascular events and thrombosis *(Ciesla, 2007).*

Factors XIIa, XIa, and kallikrein are also capable of converting plasminogen to plasmin in vitro. The

contribution of these enzymes to the activation of plasminogen in vivo is uncertain; in fact, deficiencies in these proteins do not appear to lead to pathological states that could be explained by an impaired fibrinolysis *(Moreau et al., 2005).*

Protease inhibitors regulate the contact activation of plasma. The serpins of plasma are namely C1-inhibitor (C1INH), antithrombin III, α2-macroglobulin, α1-protease inhibitor, and α2-antiplasmin. However, C1INH is the major regulator of the intrinsic system, interfering with the activities of factor XIIa and of kallikrein. Both C1INH and α2-macroglobulin account for more than 90% of the kallikrein inhibitory activity of plasma *(Moreau et al., 2005).*

2) Tissue Kallikreins

a) Nomenclature:

The Human Genome organization (HUGO) has proposed guidelines for gene nomenclature. Based on the guidelines of HUGO, an international group of scientists working in the field agreed to adopt a nomenclature for the newer Kallikreins, consistent with that already defined for Kallikreins 1-3, as shown in table (1) *(Diamandis et al., 2000).*

Gene numbering starts from centromere to telomere on chromosome 19q13.4, with the exception of the three classical kallikreins for which the existing nomenclature was retained and one newly discovered gene which maps between *KLK1* and *KLK2* genes *(Yousef et al., 2000).*

According to the nomenclature suggested recently for human tissue kallikreins, the gene is referred to as *KLK* followed by the appropriate number and the protein as KLK followed by the appropriate number *(Lundwall et al., 2006).*

Table (1): Human Kallikrein Gene and Protein Nomenclature.

New gene symbol	Previous gene symbol (S)	New Protein Symbol	Other protein names / symbols
KLK1	KLK1	KLK1	Pancreatic/renal kallikrein, hPRK
KLK2	KLK2	KLK2	Human glandular kallikrein 1, hGK-1
KLK3	KLK3	KLK3	Prostate-specific antigen, PSA
KLK4	PRSS17, KLK-L1, KLK4	KLK4	Prostase, KLK-L1 protein, EMSP1
KLK5	KLK-12	KLK5	KLK-L2 protein, HSCTE
KLK6	PRSS9	KLK6	Zyme, protease M, neurosin
KLK7	PRSS6	KLK7	HSCCE
KLK8	PRSS19	KLK8	Neuropsin: ovasin; TADG-14
KLK9	KLK-L3	KLK9	KLK-13 protein
KLK10	PRSSL1, NES1	KLK10	NES1 protein
KLK11	PRSS20	KLK11	TLSP/hippostasin
KLK12	KLK-L5	KLK12	KLK-L5 protein
KLK13	KLK-L4	KLK13	KLK-L4 protein
KLK14	KLK-L6	KLK14	KLK-L6 protein
KLK15		KLK15	

KLK, kallikrein; KLK-L, kallikrein-like; EMSP1, enamel matrix serine protease 1; hGK-1, human glandular kallikrein-1; HSCTE, human stratum corneum tryptic enzyme; HSCCE, human stratum corneum chymotryptic enzyme; TADG-14, tumor-associated differentially expressed gene-14; TLSP, trypsin-like serine protease; NES1, normal epithelial cell specific 1 gene; PRSS, protease serine; PRSSL, protease serine-like *(Yousef and Diamandis, 2001).*

b) Locus and gene strcture:

The organization of the human kallikrein locus has been extensively described in several reviews; the locus spans approximately 300 kb on the long arm of chromosome 19 in the cytogenic region 13.4 (fig.2) *(Yousef et al., 2000).* The *KLK* genes are bound centromerically by the testicular acid phosphatase gene (*ACPT*) and telomerically by Siglec-9, these flanking genes have no structural or functional relationship to the human kallikreins *(Paliouras and Diamandis, 2006).*

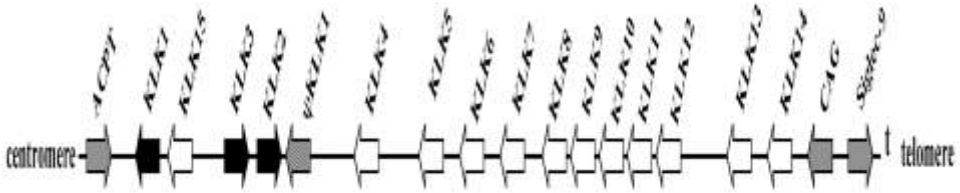

Figure (2): Human tissue kallikrein gene locus at chromosome 19q13.4. Arrowheads indicate the location of genes and their direction of transcription. Black arrowheads: the classical kallikreins KLK1-KLK3; white arrowheads: the remaining 12 KLK encoding genes: striped arrowhead: the KLK1 pseuodogene; gray arrowheads: non-kallikrein genes ACPT, CAG and Siglec9. The official gene names are indicated above each arrowhead. The orientation is shown centromere to telomere *(Paliouras and Diamandis, 2006).*

The *KLK* genes are tightly grouped and arranged tandemly without any intervention by any non-*KLK* genes. The three classical kallikreins and *KLK15* are

clustered in a 60-kb region, followed by the pseudogene C*KLK1*, and the 11 other *KLK* genes with the direction of transcription of all genes from telomere to centromere, with the exception of *KLK3* (*PSA*) and *KLK2* ***(Paliouras and Diamandis, 2006).***

All the *KLK* genes share many common characteristics, including: 5 coding exons, similar or identical coding exon lengths, and a number of splice variants and/or alternative transcriptional start sites (With the exception of *KLK14*, all kallikreins have at least one alternative transcript, exclusive of their reference form, with *PSA* followed by *KLK13* having the highest number of alternative transcripts). All kallikrein proteins are synthesized as pre/propeptides with a signal peptide of 17-20 amino acids at the amino terminus, followed by an activation peptide of 4-9 amino acids, followed by the mature (enzymatically active) protein. KLK proteins share an overall aminoacid sequence identity of 40–80% and most, if not all genes are under steroid hormone regulation ***(Kurlender et al., 2005).***

c) Members of the tissue kallikrein family: