CHAPTER I

I. INTRODUCTION

Cancer is a disease involving heritable defects in the cellular control mechanisms that result in the formation and invasive tumors capable of releasing cells that can spread the disease to distant sites in the body (Karp, 2002).

Cancer is often perceived as a disease that strikes for no apparent reason. Anyway many causes of cancer have already been identified three main categories factors that contribute to the development of cancer: chemicals (e.g. from smoking or diet), radiation and virus or bacterias. Cancer trends to involve multiple mutations and often arises because of the accumulation of mutations involving oncogenes, tumor suppressor genes and DNA repair genes.

The cancer cells

Cancer tissue has a distinctive appearance under the microscope (Figure 1). Among the traits the doctor looks for are large number of dividing cells, variation in nuclear size and shape, variation in cell size and shape, loss of specialized cell features, loss of normal tissue organization, and a poorly defined tumor boundary (Kleinsmith *etal.*, 2004).

Microscopic examination of a biopsy specimen will sometime detect a condition called "hyperplasia" which refers to tissue growth base on an excessive rate of cell division and leading to a larger than usual number of cells. Nonetheless, cell structure and the orderly arrangement of cells within the tissue remain normal, and the process of hyperplasia is potentially reversible. Hyperplasia can be a normal tissue response to an irritating stimulus. In addition to hyperplasia, microscopic examination of a biopsy specimen can detect another type of noncancerous condition called "dysplasia". Dysplasia is an abnormal type of excessive cell proliferation characterized by loss of normal tissue arrangement and cell structure. Often such cells revert back to normal behavior, but occasionally, they gradually become malignant. The most severe cases of dysplasia are sometimes referred to as "carcinoma in situ". In Latin, the term "in situ" means "in place", so carcinoma in situ refers to an uncontrolled growth of cells that remains in the original location.

However, carcinoma in situ may develop into an invasive, metastatic malignancy and, therefore, is usually removed surgically, if possible (Kleinsmith *etal.*, 2004).

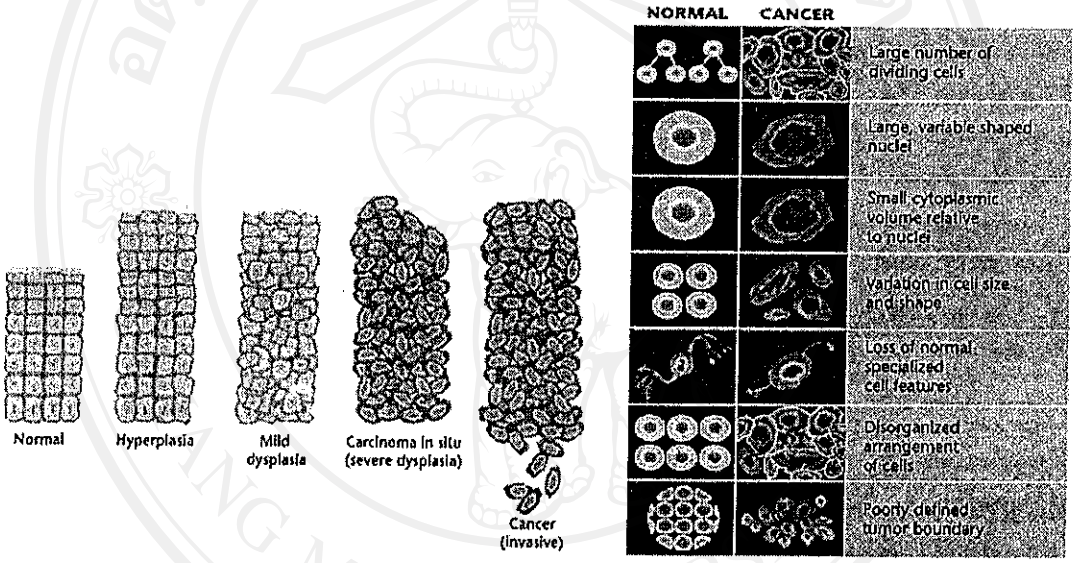

Figure 1. Microscopic appearance of cancer cells

(http://press2.nci.nih.gov/sciencebehind/cancer/cancer01.htm)

Biochemistry of the malignant cells (Duffy, 1998)

Tumor cells had a high rate of glycolysis, due to over expression of enzyme hexokinase in malignant cells, and they produced lactate even in the presence of oxygen. Enzymes involved in growth (anabolism) were found to be present at greater activities than those involved in catabolism. It is now believed that the changes identified are a feature of rapid cell growth, benign or malignant, rather than the cause of such growth. The production of proteins in forms that resembled those of fetus. As these are expressed only slightly, if at all, in the normal adult cell, they are called oncofetal proteins. This is seen in fetal isoenzyme patterns in several enzymes of intermediary metabolism, and in the re-expression of growth factors, polypeptide hormones and of cell surface molecules such as carcinoembryonic antigen. These differences may influence the behavior of the cell and its relation to other cells as well as forming the basis of many tumor marker assays.

Tumor marker (Pannall and Kotasek, 1997)

Tumor markers are substances that can often be detected in higher than normal amounts in blood, urine, or body tissues of some patients with certain types of cancer. Almost anything that forms part of a malignant cell or is produced by that cell, in certain situations, is useful as a marker. The main groups are structural molecules, secretion products and enzymes, and non-specific marker of cell turnover. It must be stressed that all these markers have their counterparts in non-malignant cells and that while there are some qualitative differences, current assay do not distinguish malignant from benign cell proliferation. Results must always considered in the overall clinical content.

Structural molecules: many tumor marker assays detect epitopes on structural molecules, most commonly these found on the cell surface these are common to many epithelial cells and so are of little value in identifying the tumor type. The tumor markers in this group consisted of carcinoembryonic antigen (CEA) and mucin.

Secretion products and enzymes: the tumor marker in this group have a relatively greater specificity for cell type of origin than the structural molecules do. The tumor marker in this group are consisted of alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and other secretion products such as prostate specific antigen, placental like alkaline phosphatase, etc.

Nonspecific markers of cell turnover: there are several markers, which reflect cell turnover or proliferation. Most are non-specific but have been used, alone or in conjugation with other markers, for monitoring tumors which do not have more specific markers. The tumor marker in this group consists of neopterin, thymidine kinase, tumor-associated trypsin inhibitor (TATI).

Interpretation of tumor markers

The ideal tumor marker would be detectable only when malignancy is present, be specific for the types and site of the malignancy, correlate with the amount of malignant tissue present, and respond rapidly to change in tumor size. At present, there is no marker that fulfills all these criteria. However, many causes of increased tumor marker concentrations of other than malignancy. If a particular tissue produces a particular marker, plasma concentration may increase. There are increased productions. This is may be due to benign or malignant cell proliferation, or to increased expression of marker by the malignant cell. Increased amounts of the marker enter the bloodstream. Increased vascularity, for example the inflammation enhances the passage of marker into the blood and leads to higher plasma concentrations. In malignancy, disruption of normal cell relationships and tissue bound-arises permits easier access to circulation. There is a decrease in the rate of disposal of the marker. If the marker is something that is normally secreted such as PSA is into the seminal fluid, the distorted architecture of tumor growth may disturb the normal anatomical relationship between cell and secretory passages and result in diversion of the marker into the blood stream. As many markers are removed from the circulation by liver, liver disease may result in reduced clearance and higher plasma concentration

Colon Cancer

Colorectal cancer is a common and highly lethal disease which cells in the colon become abnormal and divide without normal control or order, forming a mass called a tumor. Cancer cells invade and destroy the tissue around them. They can also break away from the tumor and spread to form new tumors in other parts of the body. Most, if not all, develop from adenomatous polyp over period of up to ten years, but only about 5% of polyps become malignancies.

In Thailand, neoplasm was the highest cause of death in 2000-2001 and the rate per 100,000 population was 68.4 or 11.5% of total (Thailand public health, 2003). Colorectal cancer

was the eighth most common cancer found in the new patients (4% of the total new cases). The incidence rate in female was higher than male (National Cancer Institute, 2002). The factors are linked to an increased change of developing colon cancer which appears to be associated with a diet with low fiber and high calories, protein and fat, especially in red meat. In addition, obesity, sedentary life styles and alcohol consumption have been implicated as potential risk factors (Deerasamee *et al.*, 1999). People with a family history of colon cancer, familial polyposis and those bowel disease, particularly ulcerative colitis and to a lesser extent Crohn's disease are at the high risk. Patient may present with symptoms referable to the bowel, with anemia due to blood loss, or with evidence of spread which metastasis is most commonly to liver (Wang *et al.*, 1994).

Several effective screening tests for colon cancer are available, including colonoscopy, carcinoembryonic antigen (CEA), fecal occult blood, and detection of altered human DNA in stool. The detection of blood in feces as a pointer to possible gastrointestinal malignancies. The aim of testing for fecal occult blood is to detect early cancer and bleeding adenomatous polyps. Specific assays for human hemoglobin are inherently more specific for colorectal bleeding than the chemical tests and sensitivity in patient with cancer ranges from 80-97%. To avoid false negative results, sample should be fresh and properly collected and at least three specimens should be test. In normal large bowel CEA present on the apical surface of mucosal cell where it increases in concentration as the cell travels up the gland crypt to be a small amount entering the blood. Most healthy people have plasma CEA concentrations of less than 2.5μg/L, and virtually all have concentrations less than 5μg/L. Higher concentrations may be found in smoker (Pannall and Kotasek, 1997). The new technologies for detection of colon cancer and adenomatous polyps such as CT colonoscopy is described as less invasive, more accurate than screening test (Levin *et al.*, 2003) but more expensive and take time. In clinical chemistry laboratory, the alkaline phosphatase (ALP) isoenzyme has been used as a marker to demonstrate changes in pathological conditions of the original organ where it is produced including malignancy (ALP is one of a tumor marker), for examples, the increase in liver ALP isoenzymes has been shown in hepatoma and biliary ALP isoenzyme , in cholangiocarcinoma *etc.* (Moss, 1982) .

II. LITERATURE REVIEW

I. Molecular characterization and isoenzymes of ALP

Genetic and Expression

Four structural genes encoding alkaline phosphatase have now been cloned and sequenced (Millan, 1986) and mapped to human chromosomes (Swallow *et al.*, 1986; Martin *et al.*, 1987) (Figure2). The tissue-non specific alkaline phosphatase gene expressed in osteoblasts, hepatocytes, kidney, early placental, and other cell is located on the short arm of chromosome 1, band p36.1-p34. Adult intestinal alkaline phosphatase is encoded by a locus on chromosome 2, band q34-q37, where are also located loci that encode alkaline phosphatase of the mature placenta and closely similar (placental-like) germ-cell alkaline phosphatase. The close association of these three loci presumably reflects their common, comparatively recent, evolutionary ancestry. These last three genes and their products have correspondingly similar sequences of bases and amino acids. These similarities are particularly marked in the cases of placental and germ-cell alkaline phosphatases, which are different by only 7-10 amino acid residues (98% homology), depending on the source. The sequence homology between the placental and intestinal isoenzymes is 86.5%, and between intestinal isoenzyme and tissue non specific alkaline phosphatases is 56.6%.

Expression of intestinal alkaline phosphatase is normally almost entirely confined to enterocytes. However, further report has confirmed the expression of small but significant amounts of alkaline phosphatase with the characteristics of the intestinal isoenzyme in kidney and shown it to be localized to the distal (S3) segment of the proximal tubule (Verpooten *et al.*, 1989), with potential benefits in the investigation of renal pathology. Trace expression of this isoenzyme has also been detected in other, non-intestinal tissue from a case of hypophosphatasia (Mueller *et al.*, 1983).

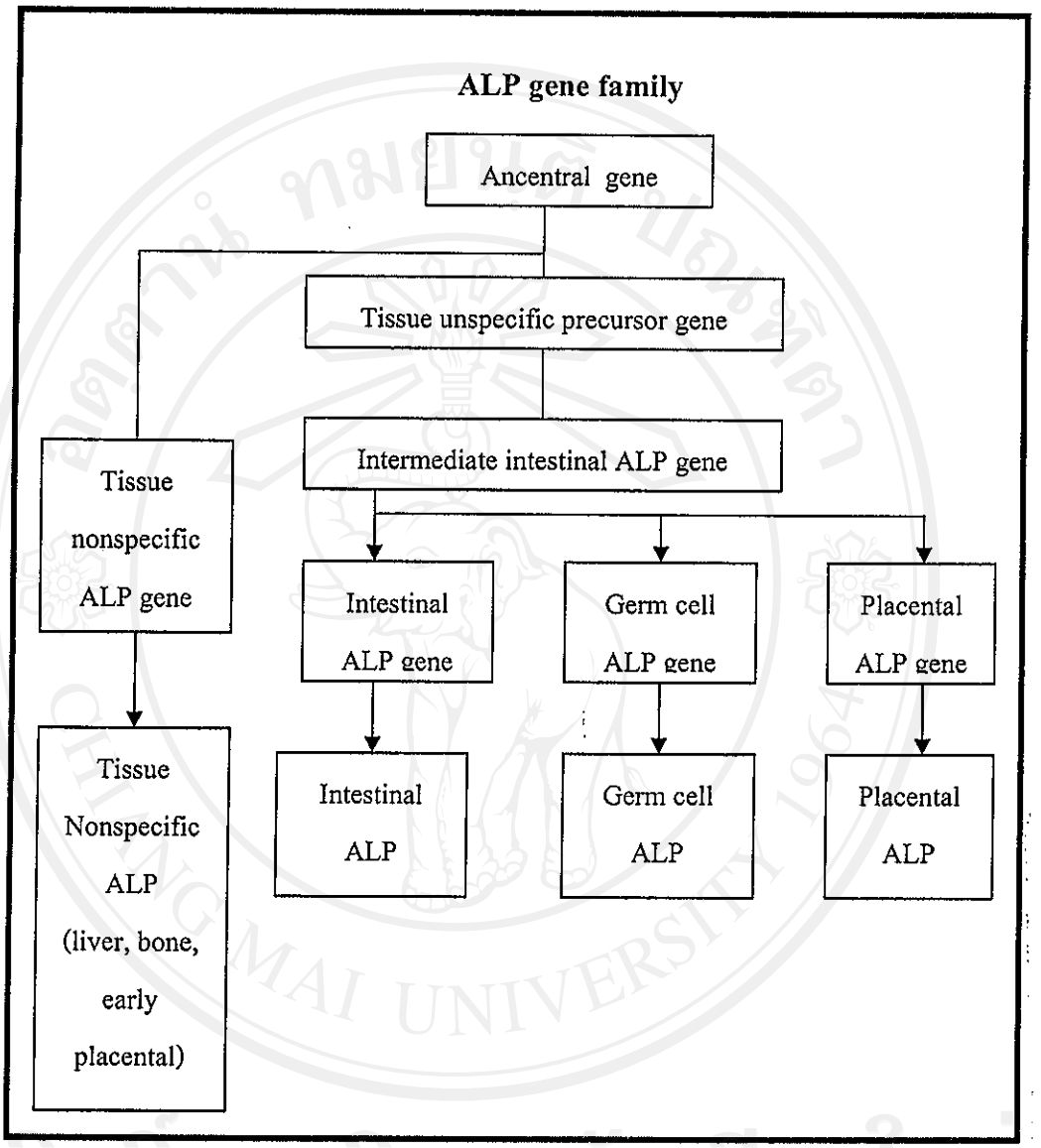

Figure 2. Evolution of ALP gene family.
(Van Hoof and De Boe, 1994)

Structure of ALP

The primary structure of human alkaline phosphatase contain a sequence of 36 amino acids which was conformationally close to the active center and absent from the enzyme of *E. coli* (Hoylaerts and Millan, 1991). Placental, germ-cell, and intestinal alkaline phosphatase show stereospecific, uncompetitive inhibition by compounds like L-phenylalanine. The action of these inhibitions depends on the residues located within this region (Hoylaerts and Millan,1991; Hummer and Millan, 1991; Watanabe *et al.*, 1991). The particular, key role is played by the residue at portion 429 in determining patterns of inhibition (Hummer and Millan, 1991; Watanabe *et al.*, 1991). This position is occupied by glutamine in placental phosphatase, by serine in intestinal phosphatase, and by histidine in the less inhibited tissue-nonspecific alkaline phosphatase. While in germ-cell alkaline phosphatase, residue 429 is glycine (Hoylaerts and Millan, 1991). Since tissue-nonspecific alkaline phosphate was the product of a single structural gene, single-residue variations in primary structure cannot account for the small, tissue-specific differences in stability to heat that were among the first differential properties of these isoforms to be identified (Moss *et al.*, 1962). The evidence from selective modification by glycosidase suggests that the differences in properties originate in differences in carbohydrate side chains (Moss and Whitaker, 1985). It is interesting to speculate whether these apparently tissue-specific differences in glycosylation are targeted specifically toward the enzyme and, therefore, by implication, have functional significance in particular cells, or whether they reflect differences in glycosylation patterns in those cells.

The structural differences between alkaline phosphatase isoenzymes were the built models of the tissue-nonspecific, intestinal and germ cell alkaline phosphatase molecules based on the 1.8A$^{\circ}$ structure of placental alkaline phosphatse (McComb and Bowers, 1985) and performed a comparative structural analysis. The examination of monomer-monomer interface at this area was crucial for protein stability and enzymatic activity. The interface allows the formation of heterodimers among IAP, GCAP and PLAP but not between TNAP with any of three tissue-specific isoenzymes. The active site cleft was mapped into three regions, i.e. the active site itself, the roof of the cleft, and the floor of the cleft. This analysis led to a structural fingerprint to the active site of each ALP isoenzymes that suggested a diversification in substrate specificity for this isoenzyme family (Le Du and Millan, 2002) (Figure3).

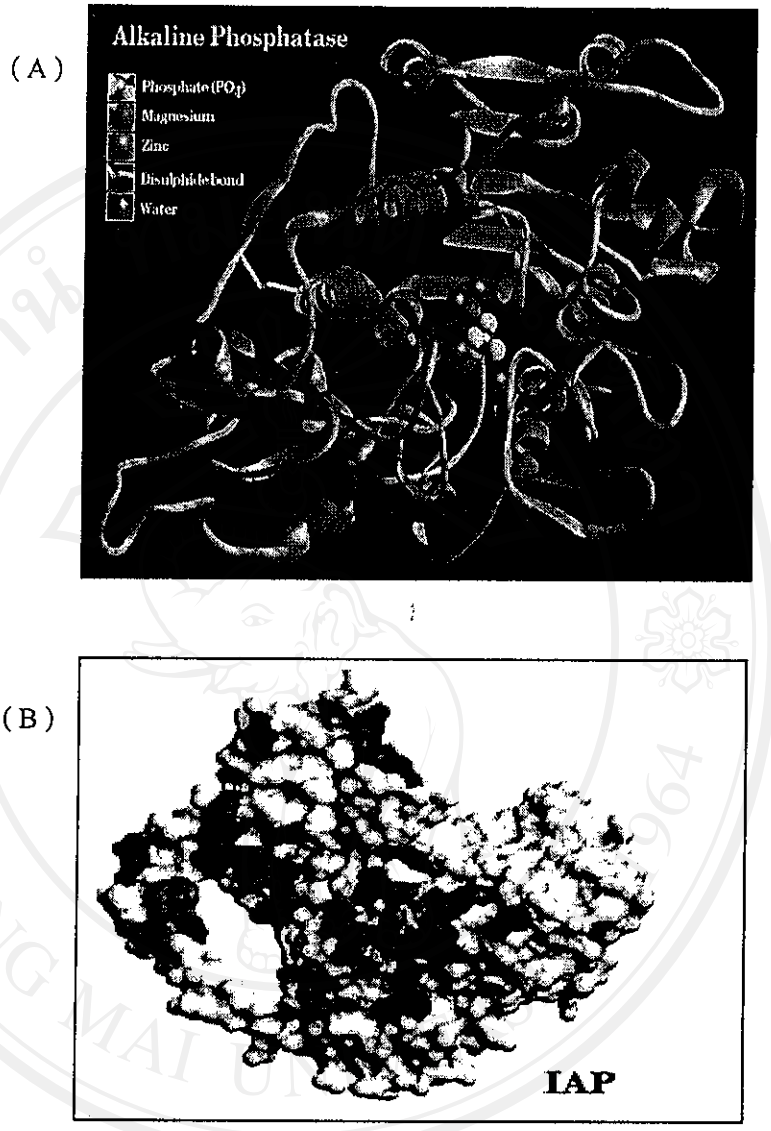

Figure 3. Structure of ALP

Ribbon drawing of a monomer of ALP (3A). The three metals are shown as stippled spheres. The monomer consists of a ten-stranded central β-sheet flasked by 15 heicles and another three stranded β-sheet and a helix on the top (Kim and Wyckoff, 1991).

Surface of IAP monomer interface involved in dimerization (3B). Residue involved a hydrogen bond or organic bond though their side chains are in red. Residues involved in a hydrogen bond or organic bond through their main chains are in blue. Hydrophobic residues involved in a stacking interaction or cation -π interaction are in violet (Le Du and Millan, 2002).

Anchoring of alkaline phosphatase to cell membrane

Alkaline phosphatase is recognized as the large group of proteins attached to outer surfaces of cells by a COOH-terminal glycan-phosphatidyl-inositol (GPI) anchor has provided into further molecular rationalization of old observations. The action of *n*-butanol is at acid pH in solubilizing tissue of alkaline phosphatase, e.g, from placenta and other different forms observed. The extraction which is effective at alkaline pH is now seen to be due to the cleavage of the GPI anchor with the activation of inositol-specific phospholipase in acid condition; the hydrophobic anchor is retained during extraction at alkaline pH (Roche and Sarles, 1954)

Glycosylation of ALP

Oligosaccharides are the integral part of these ALP isoenzymes and greatly affect their physical properties and biological functions. The glycosylation pathway occurs in the cytosol, endoplasmic reticulum(ER) (Montreuil , 1980), and the Golgi complex. It also involves transport steps including processing glycosidase, and glycosyltransferase. Protein-bound oligosaccharides are classified according to the covalent linkage between amino acid and carbohydrate. The major linkage in avian and mammalian glycoproteins are the N-glycosidic linkage between Asn and GlcNAc and three types of O-glycosidic linkage, Ser(Thr)-GalNAc, Ser-xylose, and hydroxylysine-Gal (Kornfeld and Kornfeld, 1985). Most ALPs are well known as glycoprotein which glycosylation can alter their characteristics such as electrophoretic mobility and affinity precipitation with lectin (Lehmann, 1980).

Liver, bone and kidney isoforms have distinct oligosaccharide side chains. The liver and bone ALPs show both biantennary complex-type and hybrid-type which also show asparagine linked sugar chains with and without the internal fucose residue. The bone ALP possess many more hybride-type sugar chains than the liver ALP does. Both isoforms shows different sialylation at the glycosylation end (Koyama *et al.*, 1987).

The oligosaccharides of intestinal ALP is mainly a multi- and biantennary complex type (Tsumura *et al.*, 2001). The kidney also contains small amounts of intestinal ALP which exhibit a multiantennary complex type (unbound fraction) of carbohydrate side chain. Furthermore, it was reported that the oligosaccharide chains of the renal intestinal-type alkaline phosphatase were differrent from those of meconial and adult intestinal alkaline phosphatases as revealed by lectin affinity chromatography (Nishihara *et al.*, 1992). Therefore, the intestinal-type alkaline

phosphatase can be heterogeneous. As comparison with calf intestinal ALP, the glycans linked to Asn249 are shown to have at least eight different, mainly non-fucosylated, biantennary or triantennary structures with a bisecting N-acetylglucosamine. The glycans linked to Asn 410 are a mixture of at least nine different, mainly tetraantennary, fucosylated structures with a bisecting N-acetylglucosamine. The majority of glycans are capped by alpha-galactose residues at their non-reducing terminal. In contrast to the glycans linked to other ALP isoenzymes, no sialylation was observed (Bublitz *et al.*, 2001).

Isoenzymes and isoforms of ALP presented in sera

Liver isoforms (liver ALP) mainly circulated in human sera as a soluble homodimer of two Mw 80,000 subunits (Bailyes *et al.*, 1987). Frequently, an increased liver fraction is accompanied by a so-called high molecular mass liver ALP isoform (high-Mw ALP) (Millan, 1988). This isoform has also been called fast-liver ALP (Moss, 1982), koinozyme (De Broe *et al.*, 1975), or bile ALP. It consists of liver ALP anchored to fragments of the liver cell membrane, showing variously sized vesicles by electron microscopy (De Broe *et al.*, 1985). Although ALP most frequently circulate as free enzymes, they can in some instances be complexes with other serum components, for example, lipoproteins and particularly lipoprotein-X in severe cholestasis (Brocklehurst *et al.*, 1978; Koett *et al.*, 1979: Brocklehurst 1981; Crofton and Smith, 1981; Van Hoof *et al.*, 1988).

Bone isoforms (bone ALP) mainly circulate in the soluble dimeric form, like liver ALP. In the study of healthy children, apparently of bone origin, of more than 99% in their sera (Van Hoof *et al.*, 1990). This fraction was originally called bone variant ALP and was also present in sera from adults suffering from bone disease. Like the liver variant, this fraction represents a hydrophobic heterogenous from of bone ALP. In contrast to serum membrane bound (high Mw) liver ALP and membrane-bound bone ALP in human serum is extremely rare.

For intestinal isoforms, the predominant from of IAP in the serum from healthy individuals is not the soluble dimeric IAP, but a hydrophobic, heterogenous intestinal variant ALP (Van Hoof *et al.*, 1989). The finding of membrane -bound IAP in human serum is extreamly rare. On the other hand, vesicles with a very high activity of membrane bound IAP are present in normal duodenal fluid (De Broe *et al.*, 1977; Deng *et al.*, 1992).

Placental isoforms (placental ALP) also circulates in two forms in human serum: soluble dimeric placental ALP and a hydrophobic heterogenous form (apparent Mw 440 kDa) (Hendrix *et al.*, 1990) provisionally called placental variant ALP. Because placental ALP is a very polymorphic enzyme, the presence of different allelozymes results in minor differences in its electrophoretic mobility.

II. Physiological function of alkaline phosphatase

ALPs are widely distributed in nature; they are present in all species from bacteria to men (McComb *et al.*, 1979). This is indicated that the enzyme is involved in fundamental and biochemical processes. The possible clues to its function are the GPI attachment of ALP to the cytoplasmic membrane, the uncompettitive inhibition properties of ALP, and the extracellular matrix-binding domain of ALP.

ALP in bone formation, the role of bone ALP plays in skeletal mineralization (Robison, 1923). Bone ALP activity is present on the plasma membrane of osteoblasts. Functionally active shed plasma membrane fragments of the osteoblast, and the initial site of hydroxyapatite crystal formation in bone matrix vesicles, the bone ALP activity can be much as 20 times more than osteoblast (Ali, 1986; Morris *et al.*, 1992). The following roles were postulated for mineralization process. The striking hydrolysis of orthophosphates by alkaline phosphatase at strongly alkaline pH in vitro is probably irrelevant to its function in vivo while the comparable rates of hydrolysis of pyrophosphates and orthophosphatases is at physiological pH (Moss, 1969). Osteoblastic alkaline phosphatase might favor bone mineralization by removed inhibitors of crystallization such as inorganic pyrophosphate (Coleman *et al.*, 1988). A generalized function of ecto-alkaline phosphatases in facilitating by dephosphorylation, and the entry into cells of metabolites to which the cell membrane would otherwise be impermeable and it seems a reasonable hypothesis. It is also possible that the active site of alkaline phosphatase, and also other specific modifier-binding sites indicate a receptor function for membrane-bound alkaline phosphatase. Tissue-non specific alkaline phosphatase is able to bind extra matrix proteins such as collagen (Fedde and Whyte, 1990). This may provide a mechanism for the attachment of osteoblasts to cartilage, and promoting calcification.

Liver ALP is expressed at the sinusoidal and, to a lesser extent, biliary pole of the liver cells (Hagerstrand, 1975). Although liver ALP generally represents half or more than half of serum total ALP activity in healthy adult, no precise function can yet be attributed to this isoform. The way in which ALP is anchored to the liver cell membrane might offer a clue to its physiological role in this particular organ. However, the gross deficiency of liver isoenzymes in the congenital hypophosphatasemia does not appear to give rise to any obvious clinical manifestation, therefore, it is doubtful that ALP fulfills a key function in the human liver.

Embryonal development and cell differentiation of germ-cells ALP (GCAP) may be able to interact with extra cellular matrix proteins and then serve as the cell guidance molecule during migration of germ cells down to the genital ridge (Millan, 1990). Ligands involved in directing cell migration via ALP binding, might be phosphoproteins respresented as the natural substrates ALP (Millan, 1992).

Transport of IgG molecules of placental ALP during pregnancy (Langman *et al.*, 1966), placental ALP has the potential to act as Fc receptor which is necessary to provide the fetus with maternal IgG during gestation through the acquired passive immunization.

Membrane bound ALP plays the major role in the regulation of a phosphorylate and low-conductance chloride channel in human pancreatic duct cells. Mutation of these channels involved in the pathogenesis of cystic fibrosis (Becq *et al.*, 1993).

ALP is one of the main enzymes present in the brain microvessels (Van Hoof and De Broe, 1994), which may have a role in the metabolism of PLP including a cofactor of enzymes such as glutamate decarboxylase and glutamate transaminase in the metabolism of neural tissue (Rej, 1987). Hence, ALP might be a key enzyme of the blood-brain barrier regulated by insulin (Catalan *et al.*, 1988). The ALP activity associated with capillary endothelial cells was clearly affected by a hypoxic environment and it also was significantly reduced or absent in areas of hypoxic skeletal and cardiac muscle (Hansen-Smith *et al.*, 1992).

ALP is not a Pi transporting enzyme analogous to ATPase in Na+ transport. Actually, the role of ALP in the regulation of intestinal and renal phosphate transport is indirect (Tenenhouse *et al.*, 1980). In the kidney, ALP activity is highest in early proximal tubule S1 segment, which coincides with the region of the proximal tubule where Pi transport is the highest. The luminal brush border membrane (BBM) transport of Pi and ALP activity was changed in

paralle and the positive correlation are found between the BBM transport of Pi and the activity of ALP (Dousa and Kempson, 1982). ALP may also play a role in the long-term adaptation of BBM transport of Pi to variation in dietary Pi (Kempson *et al.*, 1979). The function of ALP in the intestinal epithelial transport of Pi and calcium is a controversial subject because the use of ALP inhibitors failed to inhibit Pi transport by the intestinal cells (Moog and Glazier, 1972).

Role of intestinal ALP in lipid transport

For the role of ALP in the regulation of lipid transport, a single fatty meal also induced and increased in IAP activity human serum (Langman *et al.*, 1966). The magnitude of this response is dependent on fatty acid chain length (Glickman *et al.*, 1970). The postprandial rise in serum IAP activity was significantly greater after following a long-chain fatty acid meal than a medium-chain fatty acid meal in healthy humans under the physiological conditions (Day *et al.*, 1992). The correlation was found between lipid concentration and IAP activity in human lymph, which led to the speculation that IAP might be involved in lipid transport (Keiding, 1964).

III. Age and sex distribution of Intestinal ALP in serum

Small quantities of intestinal ALP were present in serum. At fasting state, normal activity of IAP in normal serum was approximately 25%. There are no significant differences for these isoenzymes, between age groups and between sexes. The IAP activity rises following the ingestion of a fatty meal. The presence of intestinal ALP is more frequent in sera of healthy individuals with blood group B and O who are secretors (Bamford *et al.*, 1965; Langman *et al.*, 1966; 1968).

IV. Intestinal ALP in normal and diseases

Intestinal alkaline phosphatase (IAP) is present in the apical microvilli of brush border of the enterocytes (Hanna *et al.*, 1979). Isoenzyme analysis has shown that a small amount of intestinal alkaline phosphatase is a component in normal sera of about 25%. The presence of these isoenzymes are more probable in individuals of B or O blood groups who are secretors positive and the concentration of intestinal alkaline phosphatase in their serum increases in those individuals in whom this occurs (Moss, 1973; Matsushita *et al.*, 1998). The high molecular mass

intestinal alkaline phosphatase (HIAP) and normal molecular mass intestinal alkaline phosphatase (NIAP) were found in sera of normal individuals at fasting and after fatty meals. HIAP only appeared in sera of Lewis blood group secretors [Le (a-b=)], and HIAP levels were dependent on ABO blood groups. Among the secretors, the highest activities of HIAP in fasting sera were observed in subjects with blood group O and B and the lowest activities were associated with blood group A, while the HIAP activities were not changed after fatty meals. In contrast, NIAP was present in the serum of both secretors and non-secretors regardless ABO blood group. Trace amounts of NIAP were the remaining ratios of NIAP activity in fasting serum; however serum NIAP activities rose sharply after fatty meal. The remaining ratios of NIAP activity at fasting and 9 hr after fatty meals of secretors were approximately the same as those of non-secretors (Matsushita *et al.*, 1998). Large amounts of intestinal alkaline phosphatase entered the blood circulation of all individuals by the thoracic lymph (Keiding, 1964). The persistence in small amounts in the plasma of some individuals and absence in the others suggests the difference in the rate at which the isoenzymes are removed from the blood circulation. Intestinal alkaline phosphatase is greater bound by erytrocytes of group A while it is bound to a lasser degree by those of group B or O (Bayer *et al.*, 1980). Blood group antigens are present in the intestinal phosphatase molecules, as shown by the ability of individual preparations of the isoenzymes to react with anti-blood group antisera (Komoda and Sakagishi, 1978; Komoda *et al.*, 1981).

The concentration of intestinal alkaline phosphatase in serum is increased in a variety of disease. Sometimes, it appears as the predominant isoenzymes. An increased incidence of isoenzymes has been reported in various diseases of the digestive tract (Dent *et al.*, 1968), and in other diseases, such as cirrhosis of the liver (Stolbach *et al.*, 1967; Domar *et al.*, 1988), chronic hemodialysis (Fishman *et al.*, 1965; Walker, 1974). However, the isoenzymes are not invariable present in serum in some particular conditions presumably partly because of the association with blood-group status which has already been mentioned and the preventing from of diagnostic interpretation. Nevertheless, the recognition of the possible presence of intestinal alkaline phosphatase is important for correct determination of the origin of hyperphosphatasemia (Moss, 1982).

An increased prevalence of intestinal alkaline phosphatase isoenzyme was observed in fasting sera of diabetic patients (Griffiths and Black, 1987; Tibi *et al.*, 1988), with no difference

between types 1 and 2 diabetes mellitus (Tibi *et al.*, 1988). Human intestinal ALP is completely resistant to the digestion by neuraminidase (sialidase; EC 3.2.2.18) as detected by cellulose acetate electrophoresis where as the other forms usually found in human sera are converted by this treatment to variants that migrate more cathodically on cellulose acetate eletrophoresis (Komoda *et al.*, 1981). One component of enzyme extracted from intestinal mucosa was converted by neuraminidase (Griffiths *et al.*, 1985), although this variant was a minor fraction of the small intestinal mixture, it was predominant in the colon (Griffiths *et al.*, 1992). The ALPs extracted from ilium was different from those of duodenum due to N-terminal amino acid sequence but they are similar in anti-genically and nearly identical electrophoretic mobility (Bowers and Mc Comb, 1975).

V. Intestinal ALP in carcinoma

Evidences of the increase in IAP activity in colon cancer cells have been reported by many literatures. In the study of modulation of alkaline phosphatases in Lo Vo, a human colon carcinoma cell line was shown that LoVo produced two alkaline phosphatases: the heat-labile (L-homoarginine-insensitive) of intestinal formwhich was the characteristic of its tissue of origin and the heat-stable of term-placental form which was ectopically produced by a variety of tumor (Herz, 1989). Likewise, the secretion of IAP from the ilium-like human carcinoma cell line CaCO-2 had been shown to be bidirectional-occuring at both the predominant–secreting basolateral and apical cell surfaces (Sussman *et al.*, 1989).

The *in vitro* model of the molecular events of the regulating differentiation of human colon mucosal was also reported (Celano *et al.*, 1993). The effects of Ha-ras gene overexpression on the different phenotype in human colon cancer cells were performed by expressing the v-rasH oncogene in CaCO2 cells. This maneuver resulted in a marked induction of gene expression of multiple markers which was the characteristic of the intestinal brush border differentiation. These included an equal or more than 30-fold induction of surcease, a 10-fold increase in intestinal alkaline phosphatase, a 20-fold induction of transforming growth factor alpha, and a 5-fold increase in transforming growth factor beta 1 steady-state mRNA levels.

In patients with neoplastic or hyperplastic lesions, activities of alkaline phosphatase, maltase, and dipeptidyl peptidase IV in colonoscopic biopsies from the proximal and distal colon

and rectum in 17 patients with large bowel adenomas, 29 with carcinoma, and 9 with hyperplastic polyps were compared with normal tissue (Harmenberg *et al.*, 1991). The results showed that there were a descending corectal gradient of alkaline phosphatase activities and an ascending gradient of maltase activities ($p < 0.001$) in normal control. Though, regional patterns of expression were generally preserved in disease groups, there were significant differences of alkaline phosphatase activities with patient groups (greater in cancer, adenoma, and hyperplastic groups than in normals; $p < 0.05$). For dipeptidyl peptidase IV, it was greater in hyperplastic polyp group than normals and greater in adenoma than cancer group; $p < 0.05$. Comparison with normal controls, abnormalities of site-specific activities was confined to the rectum in patients with adenoma. The maltase was decreased with $p = 0.02$ while the dipeptidyl peptidase IV was increased with $p < 0.01$ in carcinoma patients, the alkaline phosphatase was increased with $p = 0.03$ but the dipeptidyl peptidase IV as increased in all regions in bowels bearing hyperplastic polyps with $p < 0.01$. These data suggest that neoplastic and hyperplastic foci occur naturally in large bowel epithelium diffusely abnormal in terms of its expression of these enzymes.

In a case of gastric carcinoma, a variant alkaline phosphatase isoenzyme could be detected after separation of ALP isoenzymes in serum samples by polyacrylamide gel electrophoresis incorporating wheat germ lectin. Isoenzyme separation pattern showed a markedly increased amount of bone isoenzyme, a normal amount of liver isoenzyme and a considerable amount of intestinal-like isoenzyme running cathodic to the bone isoenzyme. There were also some immunoglobulin-complexed alkaline phosphates showed more number of the intestinal-like isoenzyme when it was digested (Jenkins *et al.*, 1999).

Human intestinal alkaline phosphatase can be released by enterocytes, colon cancer cells or cell line models as well as gastric carcinoma cells. The activity of enzyme was increased in differentiated colon cancer cells as comparison with normal control. There was a report that at least three isoforms, hydrophillic, hydrophobic dimers and more complex hydrophobic IAP structure presented in serum samples (Deng *et al.*, 1992). This study tried to compare the biochemical properties and characterize different isoforms of the enzyme found in sera of normal and patients with colon cancer including isolated from mucosa of colon cancer and the corresponding normal mucosa. The advantage of this study was to evaluate some laboratory technique for the early detection of colon cancer in suspected patients.

III. OBJECTIVES

1. To compare the biochemical characterizations and properties of intestinal alkaline phosphatase isoenzymes in sera of normal and patients with colon cancer.
2. To compare the biochemical characterizations and properties of intestinal alkaline phosphatase isoenzyme in sera of patients with colon cancer tumor presence and the corresponded normal mucosa obtained by colonoscopy.
3. To apply and develope a laboratory technique for screening of colon cancer disease.