Expression of the intestinal-type Alkaline Phosphatase (Iaph) in normal and malignant human tissues

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Abstract

Aim: Alkaline phosphatases (APh) are enzymes that are actively involved in important biological processes, including cell proliferation, cell-cell and cell-matrix interactions, and cell differentiation.

Materials and Methods: We studied the expression of the intestinal type AP (I-APh) in a series of normal and malignant human tissues of breast, lung, colon, head and neck, uterus, cervix, urinary bladder and prostate origin.

Results: The enzyme was strongly expressed in all cellular constituents, i.e, the epithelium, the stromal fibroblasts and the endothelial cells, of all normal tissues studied, save glandular endometrial cells. Such a consistent I-APh expression, however, was not seen in malignant tumours. In these tissues, an apparent down-regulation of I-APh expression was a feature for tumor cells, stroma fibroblasts and vessels.

Conclusions: The reason for this enzymatic suppression is far from clear. Nonetheless, the discordant I-APh phosphohydrolytic activity between normal and tumor tissues could be useful for the development of cytoprotective and/or cytotoxic compounds of high selectivity.

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Introduction

Alkaline phosphatases are important enzymes involved in several biological processes including cell proliferation, cell-cell and cell-matrix interactions, and cell differentiation [1,2,3,4]. Gevers et al recently suggested that the enzyme defines response of polycystic liver disease to somatostatin analogues [5]. At least, four types of alkaline phosphatases (APh) have been recognized, namely the liver-bone (LB-APh), the intestinal type (I-APh), the placenta germ cell type and the placenta-like type. The first is encoded in the chromosome 1, while the remaining three are encoded in chromosome 2 [6].

In lack of specific antibodies available, the expression of I- and LB-APhs in normal and malignant tissues is unknown, as yet. A polycloncal Ab against the intestinal type APh is available by Abcam (Abcam, Cambridge, UK) while, to our knowledge, no commercially available Abs against the LB-APh exist.

During the last decades, amifostine, an important cytoprotective agent has been introduced in clinical oncology providing important protection of normal tissues against platinum chemotherapy and radiation toxicities [7,8]. Amifostine (WR-2721) is an organic triphosphate that is rapidly hydrolised to its active thiolic form (WR-1065) by tissue alkaline phosphatase activity, immediately following intravenous or subcutaneous injection of the drug. This hydrolysis is performed by two major isoenzymes of APh, namely the intestinal and the liver-bone type, while placenta germ cell and placenta-like types cannot catalyze such a reaction [9].

A large body of experimental data suggest that amifostine selectively protects normal tissues and not tumoral tissues [10]. Although it has been suggested that down-regulation of APh in the tumor environement accounts for amifostine selective activity, there are no studies available examining the differential expression of the amifostine hydrolyzing APh isoenzymes in human tissues. Development of easily performed immunohistochemical test to assess I-APh and LB-APh expression in human tissues would be of importance to prove such a hypothesis.

In the present study we developed and optimized an immunohistochemical method for the assessment of the I-APh expression in human tissues, using a commerecially available polycloncal antibody. Furthermore, we comparatively evaluated the expression patterns of I-APh in normal and malignant tissues, providing strong evidence that I-APh is down-regulated in the majority of human neoplasms.

Materials and Methods

Formalin-fixed, parafin-embedded normal and malignant tissues were retrieved from the files of the Department of Pathology, Democritus University of Thrace, and were assessed immunohistochemically for the expression of the intestinal type alkaline phosphatase (IAPh). The tissues selected are shown in Table 1.

The rabbit polyclonal Ab 7324 (Abcam, Cambridge, UK), raised against human intestine was used to immunohistochemically assess the expression of intestinal type alkaline phosphatase (I-APh). As no previous experience exists with immunohistochemistry using this Ab, we standardized the optimal concentration and method for staining paraffin sections.

Table 1: Normal and malignant tissues investigated immunohistochemically for IAPh.

Tissue Type		**No of samples**
Breast	Normal Breast	10
	Ductal Carcinoma	65
	Lobular Carcinoma	10
Lung	Normal lung	10
	Squamus cancer	60
	Adenocarcinoma	30
Colon	Normal	10
	Adenocarcinomas	50
Head and Neck	Normal mucosa	10
	Squamous carcinoma	40
Uterus	Normal endometrium	10
	Emdometrioid carcinoma	35
Cervix	Normal cervix	10
	Squamous carcinoma	12
Bladder	Normal bladder	10
	Transitional cell carcinoma	20
Prostate	Normal prostate	10
	Adenocarcinoma	20

Positive and negative control tissues.

Normal human intestinal sections were used as positive controls, while normal rabbit immunoglobulin-G was substituted for the primary antibody as the negative control, at a concentration where immunostaining of control slides gave a faint cytoplasmic staining. In addition, tissues sections from the invading front of 10 colonic carcinomas (containing the

malignant tissue and the adjacent normal intestinum) were included as control samples, in an attempt to identify a tissue sample containing at the same time a positive (normal intestinum) and a negative (tumor) control. Such a sample, could be used as a double positive/negative control for subsequent immunostaining.

Immunohistochemical technique and Ab concentration.

Three μm tissue sections were deparaffinised and peroxidase was quenched with methanol and H2O2 3% for 15 minutes. Microwaving for antigen retrieval was used (3 x 5min). The primary antibody was applied overnight at different concentrations (5μg/ml, 10μg/ml 25μg/ml, 50μg/ml and finally 100μg/ml). Following washing with TBS, sections were incubated with a secondary anti-rabbit antibody (EO353, dilution 1:100, Dako Denmark) for 30min and washed in TBS. Thirty-minute incubation with the 377 (Dako, Denmark) was subsequently used for signal enhancement and sections were again washed in TBS. The color was developed by 15 min incubation with DAB solution and sections were weakly counterstained with hematoxylin. The optimal concentration (minimum background and clear nuclear and/or cytoplasmic signal) for the staining performed was fixed to 25μg/ml. Decreasing the time of incubation with the primary Ab at 2 hours, the staining results were inferior to the overnight one. Alternative immunohistocehmical methods were also performed (not shown), but the above described method was considered as the one providing the best staining. This method was followed for subsequent staining of tissues analyzed in Table 1.

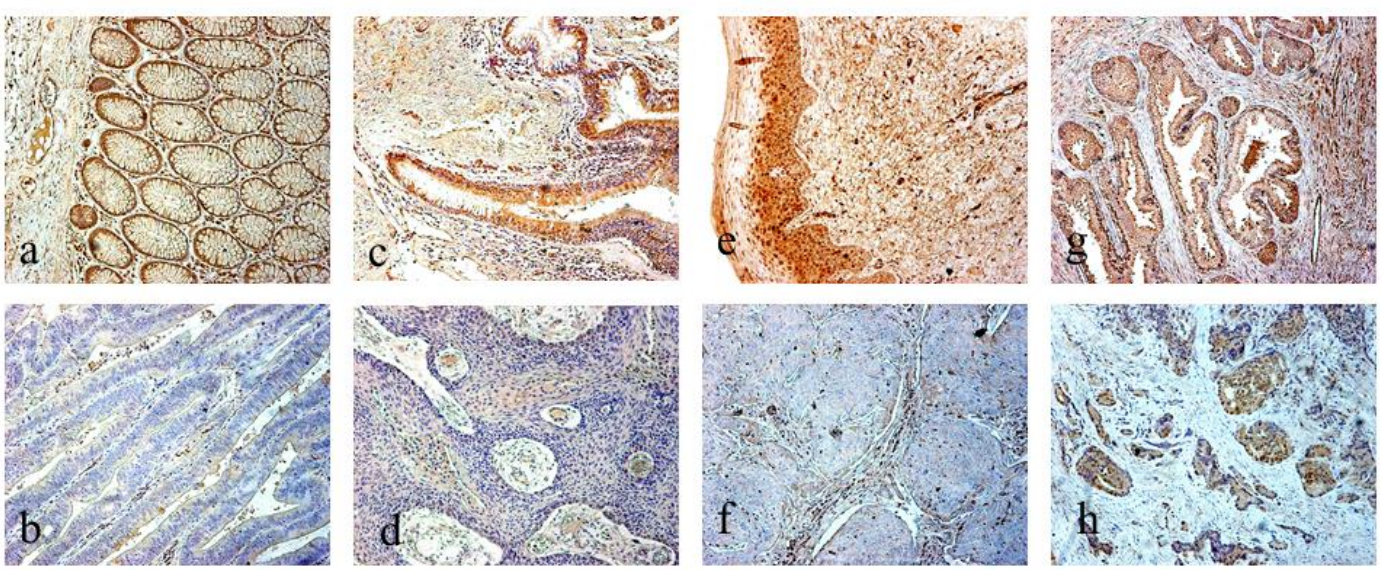

Figure 1: Intestinal type alkaline phosphatase (I-APh) is strongly expressed in normal epithelial cells, stromal fibroblasts and vessels. The expression is both nuclear and cytoplasmic (1a: intestine; 1c: lung 1e: cervix and 1g: prostate). In contrast, a striking loss of I-APh expression in tumor cells, tumor stroma and vessels is evident in malignant tissues (1b: colon cancer; 1d: squamous cell lung carcinoma; 1f: cervical carcinoma). In a minority of cases, some normal-type reactivity was maintained in a varying percentage of cancer cells, often on the background of a negative tumoral stroma (Figure 1h: prostate cancer).

Results

Positive control samples used (normal intestinum) showed a clear mixed nuclear/cytoplasmic pattern of I-APh staining of the epithelium and submucosa vessels (Figure 1a). Out of 10 samples used to identify a double positive/negative control, 6 showed a striking lack of I-APh expression in the colonic adenocarcinoma (both neoplastic epithelium and adjacent stroma cells; Figure 1b), with a simultaneous strong expression of I-APh in the adjacent normal intestinum. Tissue sections from these cases were used as controls for subsequent immunohistochemistry.

Normal breast, lung (Figure 1c), cervical (Figure 1e), urinary bladder, prostate (Figure 1g) and head/neck tissues invariably showed a strong I-APh, which was mixed nuclear/cytoplasmic in the epithelial cells and vessels and predominantly nuclear in stroma fibroblasts. Endometrial glands were persistently negative throughout the menstrual cycle.

Table 2: Analysis of the malignant tissues studied, according to the patterns of expression of intestinal type alkaline phosphatase (IAPh).

Cancer Type	No	Cancer Cell Reactivity			Stroma Reactivity	
		negative	weak	positive	negative	Positive
Breast	75	45	19	11	69	6
Lung	90	58	27	5	81	9
Colon	50	26	17	7	42	8
SCHNC	40	24	9	7	35	5
Emdometrioid	35	21	8	6	31	4
Cervical	12	8	3	1	9	3
Urinary Bladder	20	6	9	5	10	10
Prostate	20	10	5	5	15	5
Overall	342	198	97	47	292	50
(%)		57.8	28	13.8	85.3	14.7

The pattern of I-APh expression in neoplastic cells ranged from negative, weak cytoplasmic to nuclear/cytoplasmic staining (simulating the normal tissue pattern). Table 2 shows the analysis of I-APh expression patterns in tumors analyzed. The majority of cancer cases did not express I-APh. I-APh was also absent in stroma fibroblasts within the tumor environment in the majority of cases, with the exeption of bladder cancer, where strong stroma I-APh expression was noted in half of cases.

I-APh expression in tumoral vessels was rarely observed, and this was a consistent finding in all tumor examined.

Discussion

This study clearly shows that normal human tissues express I-APh in all cellular constituents, i.e., epithelial cells, stromal fibroblasts, endothelial cells and myocytes. The expression, which was invariably strong, affected both nuclei and cytoplasm. A notable exception to this trend was the glandular epithelial cells of the endometrium, which were consistently negative throughout the menstrual cycle, although normal endometrial stromal cells and smooth muscle cells were I-APh positive.

An impressive loss of IAPh expression in the tumoral epithelium and stroma was a common feature. Cancer cells were negative or showed a very-weak focal cytoplasmic expression in about 60% of cases analyzed, while a minority of cases showed a 'normal tissue type' strong cytoplasmic/nuclear expression in a varying percentage of cells. No association of IAPh expression patterns with T,N-stage and histological differentiation was noted (data not shown).

The stromal fibroblasts were negative in the majority of cancer cases. Tumoral vessels were only occasionally stained and this was confirmed by comparatively analyzing parallel tumor sections stained for I-APh and for the CD31 panendothelial cell marker. In contrast, almost all vessels stained for CD31 in normal tissues expressed a strong IAPh cytoplasmic/nuclear reactivity (data not shown).

The reason for such an intense down-regulation of I-APh expression is unknown. Genetic transformation and cellular de-differentiation may account for such an event in cancer cells [11]. On the other hand, the reason of I-APh down-regulation in stromal fibroblasts and vessels should be sought in extracellular conditions, as genetic transformation, in general, does not concern the tumoral stroma. Acidity of the cancer environment or extracellular hypoxia, which are common tumor features [12], may account for this event. Whether APh gene transcriptional regulation by the intracellular or extracellular pH or even oxygen tension occurs in mammals is unknown. Although Deren et al found that alkaline phosphatase production by mammalian periosteal cells was optimal at low oxygen tensions approximating capillary pO2, it is unknown whether up- or down-regulation of the APh genes occurs at far lower pO2 levels that dominate the tumor environment [13]. Caddick et al, reported a direct involvement of pH in the expression of alkaline phosphatase in the fungus Aspergillus nidulans, but such observation has not been reported in mammalian cells [14].

Regarding the I-APh mediated hydrolysis of the cytoprotective agent amifostine, the strong and consistent expression of I-APh in nearly all normal tissues studied ensures a high posphohydrolytic activity, which is absent in the majority of carcinomas. Amifostine selectivity can be

explained in part by the intense down-regulation of I-APh in the tumor supportive stroma. As vessels are the first tissue to interact with amifostine, the striking lack of I-APh expression in the intratumoral vessels may prevent amifostine hydrolysis within tumors. The strong expression of I-APh noted in a minority of tumors, however, cannot exclude amifostine activation in a sub-group of neoplasms. Whether I-APh expression in the tumor cells, stroma and, especially in vessels can be used as a clinical marker to confirm lack of phopshohydrolytic activity in the tumor environment requires further investigation. Such a marker could be more useful if combined with LB-IAPh expression. Certainly, these results provide a strong rational for the development of pharmacological compounds that could be activated or de-activated by the normal tissue specific I-APh phosphohydrolytic activity.

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