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Immunohistochemical and ultrastructural studies of intraluminal crystalloids in human prostatic carcinomas

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Summary. Intraluminal crystalloids (ICr) observed in 19 cases of incidental or invasive human prostatic carcinoma (PCa) and in a case of benign prostatic hyperplasia were examined extensively by immunohistochemistry and electron microscopy. They were brilliantly eosinophilic with haematoxylin and eosin, manifesting needle-like, triangular, rectangular, hexagonal and irregular lump-like in shape. They were strongly positive, dark blue, with phosphotungstic acid -haematoxylin (PTAH) stain in all cases examined. Among the human antibodies tested, epithelial membrane antigen (EMA) gave specifically positive immunostainability with ICr in all cases. Annual ring-like lamellar or concentric structures were detected by electron microscopy. Positive staining of ICr with PTAH and anti-EMA antibody is very useful as a diagnostic marker for PCa in human prostatic tissues.

Key words: Crystalloid – Prostatic cancer – Epithelial membrane antigen – Phosphotungstic acid hematoxylin stain – Ultrastructure

Introduction

Intraluminal crystalloids (ICr) in human prostatic carcinoma (PCa) have been reported as a good marker for malignancy at the light microscopic level (Jensen et al. 1980; Ro et al. 1986, 1988; Furusato et al. 1989; Monma and Sato 1991) following the first report by Holmes (1977), although they are also observed in cases of benign prostatic hyperplasia (BPH) and in glands adjacent to PCa, although at a much lower frequency (Holmes 1977; Bennet and Garder 1980; Ro et al. 1988; Furusato et al. 1989; Monma and Sato 1991). The frequency of ICr in PCa was 10–23% in routine examinations (Holmes 1977; Jensen et al. 1980; Ro et al. 1986; Monma and Sato 1991) or more than 60% in step sections (Ro et al. 1988; Furusato et al. 1989). However, the presence of ICr suggests malignancy, with few exceptions. The stainability of ICr has been reported as eosinophilic with haematoxylin and eosin (H & E) stain and there have been some fine structural studies (Ro et al. 1986). In order to characterize ICr, it will be useful to know their specific stainability but there have been no reports on special stains.

In the present study, the unique stainability of ICr with phosphotungstic acid-haematoxylin (PTAH) stain and with antibody to epithelial membrane antigen (EMA) are described, together with some interesting ultrastructural findings.

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Materials and methods

Among 78 cases of PCa and 447 cases of BPH, 19 cases of PCa and a case of BPH, known to contain numbers of ICr, were selected to investigate ICr immunohistochemically and ultrastructurally. The 19 cases of PCa were composed of 11 incidental and 8 invasive cases. The patients' ages were between 61 and 88 years. The patient with BPH was 81 years old. The prostatic tissues of each case were obtained by transurethral resection (TUR) (14 cases) or prostatectomy (6 cases).

Prostatic tissues obtained were fixed in phosphate-buffered 10% formalin solution, and embedded in paraffin after dehydration. Dewaxed 3-μm-thick sections were stained with H & E, alcian blue (AB) pH 2.5, mucicarmine (MC), toluidine blue (TB), PTAH, and dylon and congo red stains for amyloid. The sections were also tested with periodic-acid Schiff (PAS) reaction with diastase digestion (D-PAS).

For immunohistochemical staining, dewaxed sections containing ICr from each case were stained employing the avidin-biotin peroxidase complex method as reported by Furihata et al. (1992), using an SAB-PO kit (Histofine; Nichirei, Tokyo, Japan). Monoclonal or polyclonal antibodies tested are listed in Table 1. The specificity of the antibodies used has been checked by positive control sections containing various kinds of tissues and cells.

For ultrastructural examination, 10-μm-thick dewaxed sections containing ICr from 6 cases of PCa were further fixed in 3% glutaraldehyde solution containing 2% tannic acid and then in 1% osmium tetroxide solution. Each section was dehydrated through a series of graded ethanol, and embedded in an epoxy resin. Ultrathin sections were doubly stained with uranyl acetate and lead

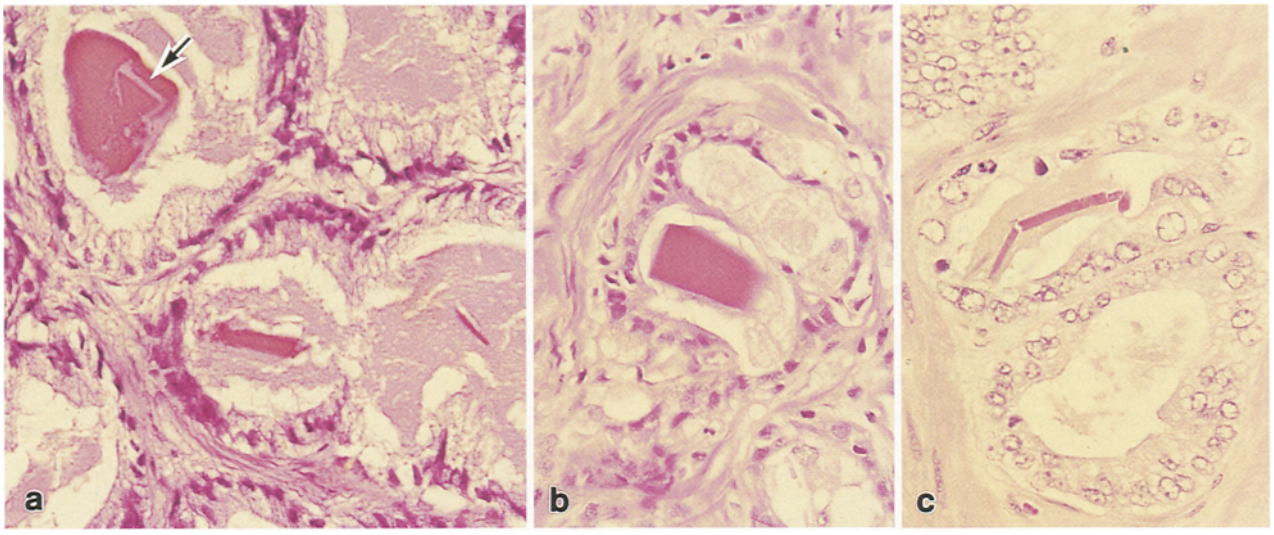

Fig. 1a–c. Needle-like (**a, c**), rectangular (b) or large lumpy (**a**) intraluminal crystalloids (ICr) are seen, some associated with light eosinophilic material (**a, c**). Note splits in a lumpy ICr (**a**, *arrow*). H & E ×240

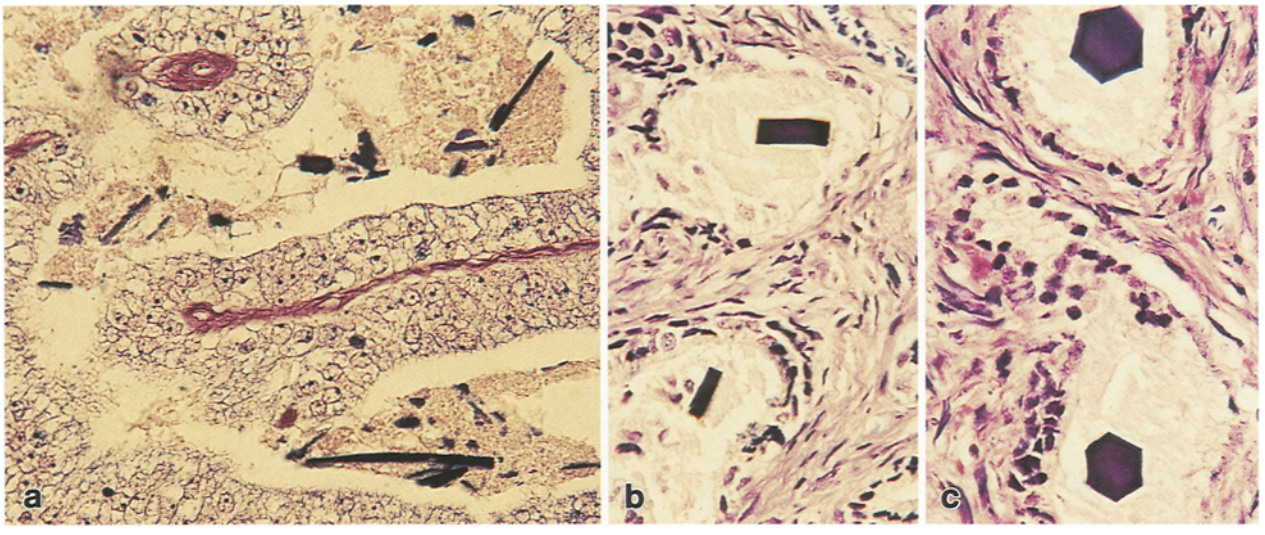

Fig. 2a–c. Needle-like (**a**), indefinite minute (**a**), rectangular (**b**) or hexagonal (**c**) ICr were positively stained with phosphotungstic acid haematoxylin in contrast with negative surrounding material. ×240

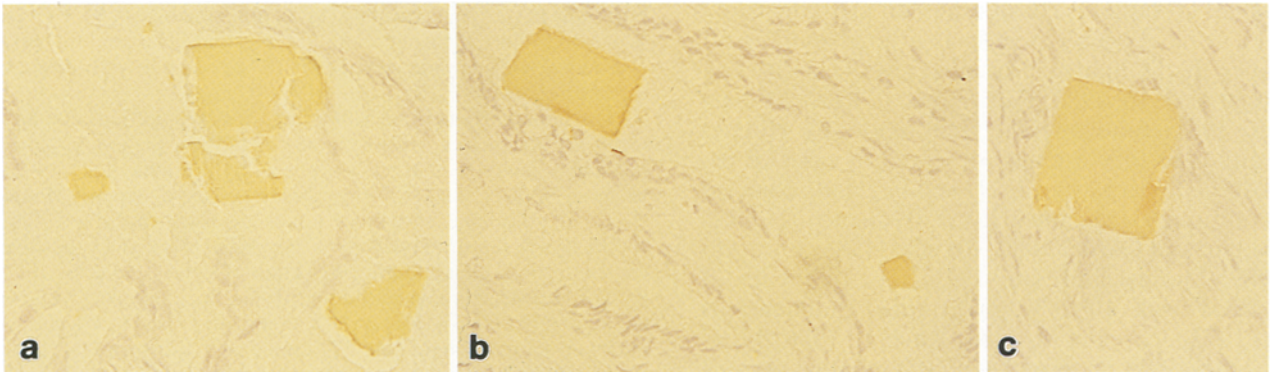

Fig. 3a–c. Antibody to epithelial membrane antigen was positive for irregular lumpy (**a**) or rectangular (**b**) ICr, even in the crushed part of prostatic carcinoma (**c**). ABC method, ×240

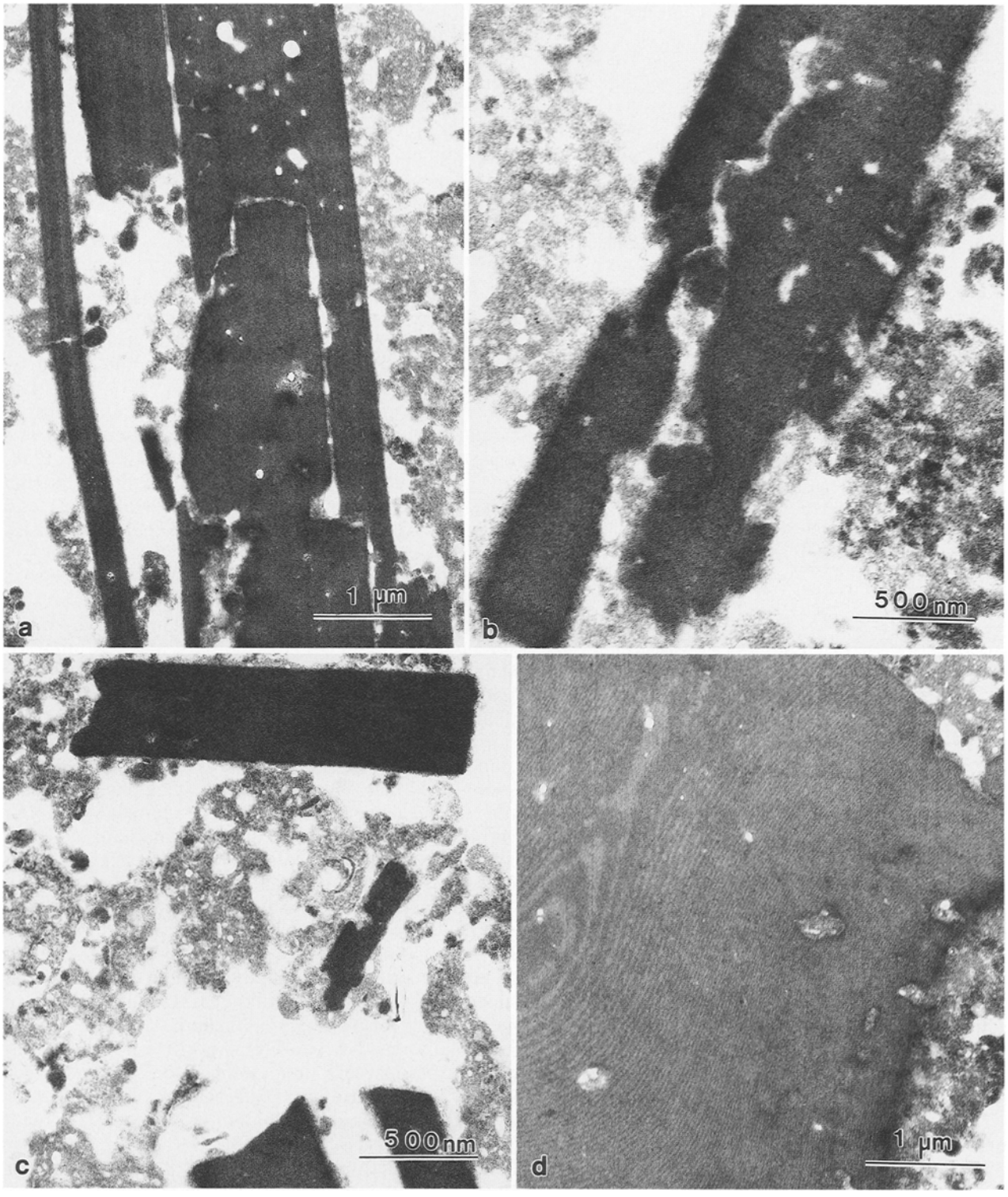

Fig. 4a–d. Electron microscopic observation shows various forms of ICr, such as needle-like ICr (**a**), ICr revealing inner splits (**a**) or marked marginal indentation (**b**), rectangular ICr (**c**) and ICr showing annual ring-like structure (**d**). **a**, **d** ×20000; **b**, **c** ×40000

citrate, and examined with an H-300 type Hitachi electron microscope.

Results

ICr were found in the intraluminal spaces of PCa, mostly incidental, well-differentiated adenocarcinomas, but rarely in BPH and in non-neoplastic glands adjacent to PCa. Generally, the numbers of ICr were decreased in clinically invasive cases of PCa. ICr, needle-like, triangular, rectangular, hexagonal, variously-sized irregular and lump-like in shape were stained strongly eosinophilic with H & E stain (Fig. 1a–c). Some were associated with light-pinkish material (Fig. 1a). The internal stuc-

Table 1. Antibodies used for immunostaining of intraluminal crystalloids in human prostatic tissues

Antibodies	Dilution	Source
Monoclonal		
EMA	1:30	Dakopatts
CEA	1:30	Dakopatts
Amyloid A component	1:10	Dakopatts
Desmin	1:30	Dakopatts
CAM 5.2	1:20	Becton Dickinson
AE1/3	1:400	Boehringer Mannheim
35βH11	1:5000	Enzo
34βE12	1:2000	Enzo
34βB4	1:400	Enzo
HHF35	1:8000	Enzo
Polyclonal		
PSA	1:200	Dakopatts
Amyloid P component	1:300	Dakopatts
β_2-microglobulin	1:300	Dakopatts
Myosin	1:1200	Dakopatts
Lysozyme	1:800	Dakopatts
α1-AT	1:800	Dakopatts
α1-ACT	1:100	Dakopatts
κ-chain	1:100	Tago
λ-chain	1:100	Tago
IgG	1:100	Tago
IgA	1:100	Tago
IgM	1:500	Tago

CEA, Carcinoembryoric antigen; EMA, epithelial membrane antigen; PSA, prostate specific antigen

tures of ICr were mostly homogeneous, but cracks or splits were occasionally observed (Fig. 1a).

Among the special stains for light microscopy performed, ICr were only positively stained with PTAH in all of 19 cases of PCa and the case of BPH, appearing dark blue (Fig. 2a–c). Needle-like ICr and rectangular, hexagonal or lump-like ICr embedded in non-stained amorphous material (Fig. 2a) were clearly identified with this stain (Fig. 2a–c), while amyloid bodies were completely negative. The PAS and D-PAS reactions, and MC and AB stains were very often positive for both amyloid bodies and also for the amorphous material surrounding ICr, but not for the ICr themselves. Positive intraluminal acid mucin was detected only in neoplastic glands of PCa, and decreased especially in widely invasive cases of PCa. The other stains performed gave the negative results for ICr.

Immunohistochemically, only the antibody to EMA gave a distinctly positive immune reaction with every shape of ICr, in all of the 17 cases of PCa examined (Fig. 3a–c), although apical regions of some glandular epithelium in both PCa and BPH showed linear positivity. ICr, even in crushed tissue, was clearly stained with anti-EMA antibody (Fig. 3c). The other antibodies tested, listed in Table 1, were completely negative for ICr, although some revealed a positive immunoreaction with intraluminal amorphous material. In the 6 cases of PCa examined ultrastructurally, ICr were needle-like, rectangular, straight or curved rod-like or irregular in form, showing high electron density with no limiting membrane (Fig. 4a–c). They were mostly less than 6 μm long and 2 μm wide, although the maximum size detected was 16 μm in length and 5.5 μm in width. They were mostly homogeneous in internal structure; however, some revealed marginal irregularities or internal slits (Fig. 4a-c) and annual ring-like structures (Fig. 4d). Approximately 1·3 nm-thick lamellae were arranged in parallel at 2·7 nm intervals in the lamellar part of the ring-like stuctures.

Discussion

When tumours increased in size following invasion, ICr appeared to be reduced in numbers, as reported by Furusato et al. (1989). ICr were mostly present in incidental well-differentiated adenocarcinomas as pointed out by Ro et al. (1988); and Furusato et al. (1989). Their sizes and shapes varied but in invasive undifferentiated carcinoma, we found that they became much smaller in size and number, as previously reported (Ro et al. 1988; Furusato et al. 1989). Similar crystalloids in the lumina were detected in struma ovarii (Ro et al. 1991) and malignant salivary gland tumours (Ro et al. 1987) with no precise characterization.

Although ICr were relatively easily detected with H & E stain, the PTAH stain was much better because of the stainability of all forms without evidence of mucin staining. PAS and D-PAS reactions, and MC and AB stains were negative for ICr, but positive for the amorphous material surrounding ICr. AB-positive acid mucin diminished in invasive PCa not containing ICr, and was not found in the glands of BPH or the non-neoplastic glands in PCa, as also reported by Ro et al. (1986). It seems likely that AB-positive mucin may play an important in the formation of ICr. The stainability of ICr with PTAH depends on an uncertain mechanism. There may be some common reactivity between ICr and fibrin or Z band material of muscle.

The presence of cross-antigenic determinants determining the positive immunoreaction of ICr with anti-EMA antibody, has not been explored. The antigen used to prepare anti-EMA antibody is a lipid extracted from human milk, and this antibody is usually thought to be a marker for glandular epithelium, although it is exceptionally reactive with CD30-positive large cell lymphoma, plasma cells or mesothelial cells. ICr was not stained immunohistochemically with antibodies to κ or λ light chain of immunoglobulin in sharp contrast with previous reports (Helmes 1977).

In fine structure, ICr were mostly homogeneous like those found in struma ovarii (Ro et al. 1991). The rare lamellar structures detected in the present study were also reported in PCa (Ro et al. 1986) Ring-like patterns, as shown in Fig. 4d, have not been previously reported.

ICr are one of the good diagnostic markers for PCa, even in crushed or distorted tissues, although not precisely specific for PCa (Furusato et al. 1989; Monma and Sato 1991). If ICr were detected in non-neoplastic glands in prostatic tissues by TUR, the possible presence of PCa should considered employing PTAH stain or immunostaining with anti-EMA antibody. Further in-

vestigation is needed to clarify the exact nature of ICr in prostatic tissue.

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