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Original Article

Immunohistochemical application of cytokeratins (#14 and #17 of Moll's cllassification), S－100β, and α－smooth muscle actin (α－SMA) as markers of myoepithelial cells to differentiate well differentiated adenocarcinoma of prostate from their related borderline malinancies

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Background : Histopathological discriminnation between well differentiated adenocarcinoma of prostate(G1－Adenoca)and its borderline mailganancies is often dificult. We investigated the usefulness of immunohistochemical identification of myoepitheliai persistence as a reliable histological criterion for benignancy, i.e. spared two－cell pattern.

Methods : Seven cases of G1－Adenoca who had been diagnosed by operatively extirpated specimens were analyzed immunohistochemically with anti－cytokerains 14(abbreviated to CK－14,presented as DAKO－34βE12) and has several foci of boderline maliganancy,i.e. atypical adenomatous hyperplasia (AAH, or adenosis)(1－3) and prostatic intraepithelial neoplasia(PIN)(4,5).These results were compared to those of several normal controls of both breast and prostate.

Results : The positive staining of CK was confirmed diffusely in cytoplasm though there was no reactivity in S－100β and α－SMA. The number of immunoreactive myoepithelial cells was in inversely proportional to degree of atypism.No myopithelial cells was identified in G1－Adenoca by immunostaining method

Conclusions : The preservation of normal basal myoepithelial cell layer is helpful to differentiate the borderline mailgnacies from the G1－Adenoca, and this is easily identified by the immunohistochemical staining with mixed reagents of CK－14 and 17.

Key words : prostate, borderline ,alignancy, discrimination, cytokeratin 17, 34βE21, S－100β, α－smooth muscle actin. immunohistochemistry

introduction

It was frequently difficult for pathologists to make the diagnosis of G1－Adenoca from small biopsied specimens.The histological diagnosis of G1 －Adenoca was very different among pathologists because the histological differentiation of G1－Adenoca from its borderline mailgnansies depended upon very subjective diagnostic criteria.(1－5)

Borderline mailgnancies consisted of two groups: (a) AAH(1,2) or adenosis(3)as a benign counterpart of G1－Adenoca of small asinar type,(b)PIN or atypical intraductal hyperplasia(4,5) or dysplasia as a benign couterpart of G1－Adenoca of large acinar type with/without papillary structure.PIN was divided into either 3 groups(I , II ,andIII)(4) or 2 groups (low－grade and PIN or high－grade PIN corresponded to PIN I or PIN II＋III, respectively, corresponding to the classification of (uterine) sqamous intraepithelial lesions(SIL)of low－grade or high－grade to cervical intraepithelial neoplasia (CIN) I or CIN II＋III. Adenosis (or AAH) showed microglandular proliferation like mastopathy and was derived from the terminal acino－ductular unit (TADU) like the intralobular

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terminal duct (ITD) of breast,i.e. peripheral portion of the terminal ductal−lobular unit(TDLU)of breast. PIN,on the other hand, revealed intraductal epitheliosis or ductal hyperplasia like mastopathy and was derived from ductulo−ductal regions like ducts and the extralobular terminal duct (ETD) of breast, i.e. proximal portion of TDLU of breast.AAH and PIN had frequently complicated each other in the same specimen because of their common origin of ductules.There have been reported many histological differential criteria between G1−Adenoca and borderline mailgnancies.(1−5)The preservation of myoepithelial cells, i.e. preservation of two−cell−layer configuration,was considered sa the most reliable discriminating factor of benignancy(1−9)(Fig.1,2).

AAH derived from TADU had very few myoepithelial cells because there were ordinarily only few myoepithelial cells in TADU. The more severe the atypism of PIN was,the fewer the myoepithelial cells were. Accordingly, among these borderline mailgnancies, it was often difficult to find these fewer myoepithelial cells in specimens stained with the hematoxylin−eosin. In this study the immunohistochemical detection of myoepithelial cells was performed with four antibodies as the markers of myoepithelial cells, including the reagants toward cytokeratins(CK), including both CK−14 (DAKO−34βE12)(6) and CK−17, S−100β, and α−SMA.

Materials and Methods

Specimens: Seven cases of G1−Adenoca diagnosed histopathologically with the operatively extirpated prostate were available for this study.Each case had several interposing foci of AAH or AAH or PIN.AAH was histologically diagnosed by the finding of acino−ductular structures with uniform columnal cells,and PIN was diagnosed by macronucleolus and the pattern of either cellular bridging or cribriform(Fig.1−3).These lesions of borderline malignancy necessarily contained myoepithlial cells regardless of their number.The immunohistochemical results were compared among normal region,AAH,PIN,and G1−Adenoca.Several breast tissues were also available for control.Routinely −processed paraffin−embedded sections were used.

Reagents:Following primary antibodies were commercially provided:1.in Moll's classification, 68kD,58kD,56.5kD,and 50kD of molecular weight, respectively,and CK−14(50kD)was specific to myoepithelial cells(DAKO−34βE12,DAKO Co.,Denmark)(Fig.4)(10),2.Monoclonal mouse anti−rat cytokeratin 17D(43kD of molecular weight)(CK−17,DAKO−E3,Dako Co.,Denmark)(Fig.4)(10),3.Monoclonal mouse anti−cow brain S−100β(Nippon Kohtai Kenkyusho,Takasaki,Jappan),4.Monoclonal mouse anti−human α−SMA(DAKO−1A4,Dako Co.,Denmark).

Procedures:Routinely immerced tissue sections were preincubated by heat trestment with a microwave.After the reaction with primary antibodies routine peroxidase−anti−peroxidase reaction was performed.

Results

Cytokeratins:In prostatic specimens,normal basal myoepithelial cells were diffusely positive in their whole cytoplasm and arranged continuously in immunohistochemical studies by both CK−14(34βE12)andCK−17.(Fig.5)The staining intensity was severe in CK−14(34βE12)than in CK−17.In both adenomatous hyperplasia(AA)and PIN I(4)(low−grade PIN)(5),however,the basal myoepithelial cells lined discontinuously.This severity of discontinuity increased in proportion to the atypism in AAH(4)and PIN II or III(high−gradePIN)(5).In G1−Adenoca,normally−stained myoepithelial cells were completely disappeared.Instead of diffusely and even immunostained myoepithelial cells,furthermore,there appeared several tumor cells that stained granularly.The intensity of immunoreaction of myoepithelial cells by each cytokeratin was sligthtly increased by the mixed reagent of primary antibodies of both CK−14(34βE12)and CK−17,i.e.so−called cocktail reagent of CKs.In breast specimens,there was no reactivity in myoepithelinm,but the positivity was found in epithelium.

S−100β:There was no positive reaction in basal myoepithelial cells of prostate like the negative

immunoreaction in those of breast.
α −SMA: There was no positivity in basal myoepithelial cells of prostate though those of breast immunoreacted. This meant that mammary myoepithelial cells had intermediate filaments of muscular actin and indicated much muscular characteristics rather than those of prostate.

Discussion

Classical diagnostic criteria of mailgnancy depending on the severity of atypism were not used for the histopathological diagnosis of G1−Adenoca but for poorly differentiated adenocarcinoma because G1−Adenoca frequenthy appeared with less atypism than that of borderline lesions. As a result, it was not easy that present differential criteria applied clinically to differentiate G1−Adenoca from bordeline lesions. (Fig.1,2) (1−5) It was reported thst adenosis or AAH was histologically diagnosed by their uniformity like normal acino−ductular configuration and PIN was diagnosed by the presence of bridging or cribriform configuration. Practically these criteria were useless because these findings were very similar to those of benign prostatic hypertrophy and specimens derived from elder patients frequetly had same lesions. The macronucleolus over 3 μ m in diameter and the loss of basal myoepithelial cells were only reliable criteria for G1−Adenoca. This reservation of myoepithelial cells was frequently difficult to identify in routinely stained specimens because there were naturally only few myoepithelial cells in acino−ductular areas and the number of myoepithelial cells decreased in proportion to PIN atypism. So it was necessary that the assisted immunohistochemical examinnation should be done to confirm these fewer myoepithelial cells.

In this study for the identification of myoepithelial cells we used four primary antibodies: (a) antibodies confirming of cytokeratins (intermediate filaments of epithelial characteristics in myoepithelial cells of two−cell−layer): CK−14 (34 β E12) and CK−17 (Fig.4), (b) antibodies confirming of Calcium−binding protein (charateristics of conducting system including myoepithlial cells): S−100 β .

Nomal basal myoepithlial cells were diffusely stained in their whole cytoplasm and there positive cells arranged continuously in immunohistochemical studies by both CK−14 (34 β E12) and CK17. The staining intensity was more severe in 34 β E12 than CK−17. In AAH (1−3) and PIN (4), the discontinuation of myoepithelial layer appeared and its severity of discontinuity increased in proportion to stypism in AAH and PIN II or III (high−grade PIN) (5). In G1−Adenoca, normally−stained myoepithelial sella were completely disappeared and there was only single−cell−layer pattern without myoepithelial cell layer.

Instead of diffuselly and even immunostained myoepithelial cells, furthermore, there rarely appeared several cancerous cells that stained granularly. The cooktail antibodies consisted of both CK−14 (34 β E12) and CK−17 increased the intensity of immunoreacton of myoepithelial cells rather that by individual reagent. There was a possibilitty that borderline mailgnancies reduced or modified the cytokeratin reactivity in myoepithelium and marked discontinuation of myorpithelial layer was regarded as high−grade PIN or AAH. This mixed reagent was thought to make up a deficit each other and it would be available for routine identification of myoepithelial cells. The muscular character of myoepithelial cells of prostate was far weaker than those of breast.

There was no positive staining in myoepithelial cells by reagents against α −SMA and S−100 β

There were, furthermore, many other immunohistochemical analyses to distingush G1−Adenoca from boderline malignanicies (6−9) (Fig.3)

G1−Adenoca reacted weakly with Ki−67 and p53 as proliferation marker or oncosuppressor products and we often failed to identify their positivity (Fig.3). c−erbB−2 oncoprotein was strongly positive in luminal cells of both PIN and G1−Adenoca, so it could not be used for differentiation. (9) The persistence of neurondocrine cells in luminal layer was valuable for benignancy (8), but there was the probability that G1−Adenoca with intreductal lateral spreading frequently swallowed up neuroendocrine cells of surrounding normal luminal cells.

The reactivity of bcl−2 oncoprotein in myoepithelial cell layer was relatively intenser in PIN than in G1−Adenoca.The amount of bcl−2 oncoprotein corresponded to the preservation of myoepithelial cells as benignancy because bcl−2 oncoprotein was regarded as apoptosis blocking protein.The staining difference between these two groups was indistinct because the intensity of staining was continuous and there was no distinct boundary between them.

The basement membrane confirmed by laminin or fibrinogen IV was used to identify the invasion of G1−Adenoca.These stainabilities were reported to be well preserved in PIN but relatively interrupted or lost in G1−Adenoca.But oppositely did G1−Adenoca sometimes induce the production of extracellular matrix to duplicate or thicken basement membrance.

A microwave pretreatment brought sensitive and amplified positivity,which decreased ambiguous reactions only because of its early poor staining techniques.After a pretreatment with microwave became a standard procedure,it was unnecesary to be troubled with the possibility of false negative reactions.

Concerning the technical terms of PIN,the prefix"P" was "name of organ" and the suffix" IN" was" intraepithelial neoplasia",which was derived from gynecological term" (uterine)cervical intraepithelial neoplasia(CIN)".This"IN"was also used for"vulvar intraepithelial neoplasia(VIN)"," endometrial intraepithelial neoplasia(EIN)","prostatic intraepithelial neoplasia(PIN)",and so on. The" IN"was used for the histopathological classification of organs containing troublesome broderline malignancies to differentiate."IN"lesions were usually classified into 3 stages,consisted of definite benignancy with mild atypism(I),suspicious mailgnancy or sometimes including early cancer,confirmed by the DNA analysis (III),and their medium(II) .IN−III were defined by the similarity of normality or early cancer,respectively.On the other hand,the criteria of IN−II was ambiguous because IN−II was defined only by the gap between IN− I and IN−III.

Recently,CIN was practically reclassified into 2 stages by Bethesda system in 1988:low−grade squamous intraepithelial lesions (SIL) and high−grade SIL. This two−step classification was very reasonable and practical because low−grade SIL was benign, and high−grade SIL tended to G1−Adenoca and sometimes included early cancer.The identification of high−grade SIL was very important because strict follow−up was recommended in high−grade cases regardless of containing early cancer.The practical boundary lesion like high−grade SIL was,to our regret,necesary to exclude a matter of opinion of pathological discrimination and a medical lawsuit.On this viewpoint the term of PIN should be classified as low−grade and high−grade.

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Fig. 1. Histopathological differentiation between AAH(adenosis)and well differentiated adenocarcinoma derived from acinoductular structures : summarized from referrences#13.

atypism/findings	AAH	carcinoma
infiltration	−	+
uniformity	−	+
enlarged nucleolus	−	+
basal cell layer	+	**−**

AAH : atypical adenomatous hyperplasia,
uniformity : no definitive atypism in glands and cells,
enlarged nucleolus : 3μm≦diameter,
basal cell layer : preservatin of 2−cell−layered structure.

Fig. 2. Histopathological differentiation between PIN and well differentiated adenocarcinoma derived from ductal structures : summarized from referrences#4, 5.

findings/histology	PIN		carcinoma
subclass-grade(Drago, '89)	low-	high-	
(Bostwick, '87)	I	II, III	
cribriform, bridging	−	±	+
uniformity	−	±	+
enlarged nucleolus	−	±	+
basal cell layer	+	±	**−**

PIN : prostatic intraepithelial neoplasia
uniformity : no definitive atypism in glands and cell,
enlargednucleolus : 3μm≦dimeter,
basal cell layer : preservation of 2−cell−layered stucture.

Fig. 3. Immunohistochemical differentiation between PIN and well differentiated adenocarcinoma derived from ductal structures listed from referrences#6−9.

histlogy	luminal cell				myo	BM
reagents	Ki-67	p53	c-erbB-2	neuroenocrine cells	bcl-2	laminin fibrinogen IV
PIN	−	−	++	↓	++	+/±
Gl-Adenoca	±	±	++	−+	+	±/−

bcl2 : apotosis blocking protein
BM : basal membrane
myo : myoepithelial cells of outer layer in two−cell−layer
neuroendocrine : existance of neuroendocrine cells
PIN : prostatic intraepithelial neoplasoa

Fig. 4. Cytokeratins and their specific localization quoted from referrence #10.

histology/type of cytokeratin	II	I	
stratified=squamous			
keratinizing	[1]-3	9,[10],11	
non-keratinizing	4,6	13,16	
			2-cell-layered
basal	[5]	[14],15,17,(19)	outer layer (myoepithelium)
monolayered	7,8	18,19,20	inner layer (epithelium)

arabic number of cytokeratin: based on Moll's classification
34 β E12 reacting cytokeratins of type [1], [5], [10], [14].
CK 14, 15, 17, (19) specific to basal layer of 2-cell-layer.

Fig. 5. Results of immunoreactivity of myoepithelial cells in this study

organ	34βE12	CK-17	α-SMA	S-100β
prostate epithelium	−/±	−/±	−	−
myoepithelium	**++**	**±/+**	−	−
breast epithelium	+	+/±	−	−
myoepithelium	−	−	++	−

CK:cytokeratin
34βE12 : including CK-14
α-SMA : α-smooth muscle actin

Immunohistochemical application of cytokeratins(#14and#17of Moll's cllassification), S−100β, and α−smooth muscle actin(α−SMA)as markers of myoepithelial cells to differentiste well differentiated adenocarcinoma of prostate from their related borderline malinancies

サイトケラチン、S-100β、α-smooth muscle actinに対する免疫組織科学的筋上皮細胞の同定による前立腺の高分化型腺癌と境界病変との識別

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前立腺癌分化型（G1−Adenoca）と、その境界病変（atypical adenomatous hyperplasia(AAH),prostatic intraepithelial neoplasia(PIN)）の病理組織学的鑑別は、通常のHE染色標本では困難なことが多い。われわれは以前よりoncosuppressorやproliferation markerであるp53やKi−67等の免疫組織学的発現は癌の判定に有効であることを示してきたが、その発現頻度は低く、いずれの症例にも応用できるものではなかった。

一方、従来より提唱されている筋上皮細胞層の消失（即ち、正常二層構造の消失）をもって癌と判定する病理組織学的基準は、正常・境界病変・癌の一連の連続する病変を筋上皮細胞の多寡により半定量的に鑑別しうると判断される。われわれは、筋上皮細胞の免疫学的同定に従来より使用されてきた34β E12（cytokeratin 14）に更に、cytokeratin 17を加えることにより、境界病変・癌症例における筋上皮細胞のcytokeratin発現異常にかかわりなく、筋上皮細胞を明瞭に同定することができた。また、免疫組織化学的手法により筋上皮細胞数の定量化により、著明な筋上皮細胞層の消失部分をAAH、high−grade PINと診断することが出来た。

キーワード：前立腺、境界病変、鑑別、サイトケラチン17、34βE12、S-100β、α-平滑筋アクチン、免疫組織化学

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