Eduardo Scortegagna

COMPORTAMENTO DAS CÉLULAS MUSCULARES LISAS NOS CARCINOMAS DA PRÓSTATA HUMANA: VARIAÇÕES FENOTÍPICAS ULTRAESTRUTURAIS

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Orientador: Prof. Dr. Hernandes Faustino de Carvalho

Co-Orientador: Prof. Dr. Sebastião Roberto Taboga

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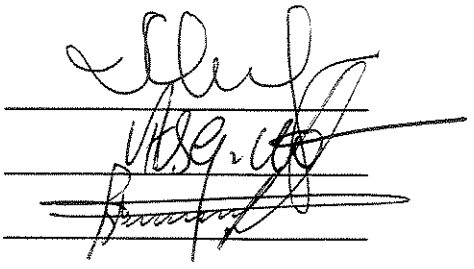
Titulares

Prof. Dr. Hernandes Faustino de Carvalho

Profa. Dra. Valéria Helena Alves Cagnon Quitete

Prof. Dr. Edson Rosa Pimentel



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Profa. Dra. Carla Beatriz Collares Buzato _____

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Resumo

As células musculares lisas da próstata humana foram estudadas ao microscópio eletrônico de transmissão, a partir de amostras obtidas por prostatectomia radical em casos de carcinoma da próstata. As células musculares lisas apresentam-se comumente formando feixes, nos quais elas aparecem intimamente associadas com as vizinhas, sendo que as membranas basais de células adjacentes mostram-se únicas. Com o desenvolvimento tumoral, nas áreas de proliferação epitelial em tumores com graus intermediários de diferenciação glandular, inicia-se um acúmulo de matriz extracelular entre as células vizinhas, sendo que as membranas basais tornam-se únicas para cada célula, refletindo a perda dos contatos homotípicos. Com a invasão tumoral, nos tumores altamente indiferenciados, as células musculares lisas apresentaram três fenótipos distintos: *atrófico, ativado* e *degenerado*. As células *atróficas* possuem uma proporção núcleo/citoplasma elevada, com notada diminuição do componente contrátil e com membrana basal menos desenvolvida e comumente interrompida. O fenótipo *ativado* mostra acúmulo de material vesicular nas regiões periféricas e intenso pregueamento da superfície celular em regiões de íntimo contato com elementos fibrilares da matriz extracelular. Em algumas células nota-se um aumento na proporção de organelas como retículo endoplasmático granular e Golgi, em detrimento do citoesqueleto. O fenótipo *degenerado* possui citoplasma bastante reduzido, com núcleo colapsado e com espaço perinuclear expandido, sendo que a membrana basal está interrompida. Uma série de prováveis conversões entre estes fenótipos das células musculares lisas é proposta. As modificações das células musculares lisas parecem decorrer da perda da sinalização proveniente do epitélio e também a partir da degradação da membrana basal por enzimas produzidas pelas células tumorais.

Abstract

The smooth muscle cells of the human prostate were studied at the electron microscopy level, from samples of radical prostatectomy in cases of prostate carcinomas. The smooth muscle cells are found in bundles, in which they are intimately associated to each other and with fused basement membrane. With the tumor progression, in the areas of glandular proliferation in the intermediary graded tumors, there is an accumulation of extracellular matrix between the smooth muscle cells, which loose the homotypic contacts and acquire individualized basement membranes. With the stromal invasion by the epithelial cancer cells, the smooth muscle cells show three different phenotypes: atrophic, activated and degenerated cells. The atrophic cells show a diminished cytoplasm/nucleus ratio, with a marked loss of the contractile component and showing a reduced and frequently disrupted basement membrane. The activated phenotype shows an accumulation of vesicular material at the cell periphery and intense folding of the cell surface in regions of intimate contact with extracellular fibrillar components. Some cells had an increase in the amount of organelles such as the rough endoplasmic reticulum and Golgi while the cytoskeleton is diminished. The cells of the degenerated phenotype have a reduced cytoplasm, collapsed nuclei and expanded perinuclear spaces. The basement membrane around these cells is disrupted. A series of conversions between these smooth muscle cell phenotypes is proposed. The modifications observed in this study seem to occur by the lack of a proper stimulation by the epithelium and/or from the degradation of the basement membrane by proteolytic enzymes produced by the tumor cells.

Introdução

A próstata humana – Aspectos anatômicos e regionais

A próstata humana circunda a uretra na base da bexiga urinária. Ela tem a forma aproximada de um cone invertido, com peso de cerca de 20 a 60g no homem adulto. A uretra prostática faz um ângulo de 35° na metade do seu percurso pela próstata (anteriormente ao verumontanum) e recebe diversas aberturas dos dutos prostáticos (Greene et al. 1995).

A próstata é dividida morfologicamente em quatro zonas principais, segundo McNeal (McNeal, 1981; Greene et al. 1995; Droller, 1997). A região de transição (1) é uma estrutura grosseiramente esférica, localizada distalmente ao ponto de angulação dentro da próstata e que corresponde a cerca de 5% do volume prostático normal. Esta região aumenta gradativamente com a idade, principalmente por conta da ocorrência de hiperplasia prostática benigna (McNeal, 1981). A região central (2) compreende cerca de 20-25% do volume prostático. Ela tem um formato cônico, sendo que a parte afilada localiza-se abaixo da região central, à qual se funde, mantendo, entretanto os limites morfológicos. Os dutos ejaculatórios percorrem a região central, em seu trajeto prostático, até a uretra. A região periférica (3) recobre as duas regiões anteriores e os dutos que dela se originam drenam para a uretra numa região proximal, antes dos dutos ejaculatórios. Finalmente, existe uma região fibromuscular anterior (4), que recobre parte da próstata. Ela é ampla na região de saída da uretra e afina-se na direção posterior, na entrada da uretra na próstata. Esta região é desprovida de unidades glandulares, corresponde a cerca de 30% do volume prostático. Esta região adere-se fortemente à porção glandular prostática.

A próstata humana – Aspectos histológicos

A próstata possui uma região epitelial, dita parênquima prostático e uma região de tecido conjuntivo, denominada estroma. A partir da uretra surgem dutos que se ramificam conforme

adentram a próstata propriamente dita e que terminam em ácinos. Os dutos são indistintos dos ácinos, no que diz respeito à morfologia das células epiteliais, mas distinguem-se por apresentarem ramificações, enquanto os ácinos são mais arredondados (Epstein, 1995).

As porções proximais dos dutos prostáticos são recobertas por um epitélio de transição, à semelhança do que acontece na uretra. Nas porções distais dos dutos e em alguns ácinos, alternam-se epitélios cuboidais, transicionais e colunares (Epstein, 1995). A ocorrência de epitélio transicional é rara, acontecendo em crianças e recém-nascidos, sendo às vezes denominado de metaplasia de células transicionais. As células transicionais tem formato fusiforme, com núcleo apresentando reentrâncias e com o longo eixo paralelo à membrana basal, formando, muitas vezes, uma camada sob as células epiteliais principais.

As células epiteliais secretoras são altas, com citoplasma claro e apresentam reação positiva para o PSA (antígeno específico da próstata) e para a fosfatase ácida específica da próstata (PSAP) e não apresentam reação para citoqueratinas de alto peso molecular (Reese et al. 1988; Hedrick & Epstein, 1989; Epstein, 1995).

As células basais ficam junto à membrana basal, abaixo das células epiteliais secretoras. Elas são fusiformes e orientam-se paralelamente à membrana basal (Mao & Angrist, 1966). Seus núcleos são ligeiramente ovalados e com cromatina frouxa. Estas células apresentam reação para citoqueratinas de alto peso molecular (Hedrick & Epstein, 1989).

Aparentemente as células basais são as responsáveis pela reposição das células epiteliais secretoras que descamam para a luz das unidades secretoras (Liu et al. 1997). Estas células basais estão envolvidas no processamento de andrógenos e na sua apresentação para as células epiteliais secretoras (El-Alfy et al. 1999).

No epitélio prostático existem ainda células com diferenciação neuroendócrina, que formam populações isoladas. Elas são células que apresentam reação com a prata (Epstein, 1995). A natureza neuroendócrina destas células é confirmada pela reação com anticorpos contra serotonina, hormônio adrenocorticotrófico (ACTH), calcitonina, e enolase específica de neurônios (DiSant'Agnese, 1992). Histologicamente, elas são reconhecidas por apresentarem grânulos pequenos e basais intensamente eosinofílicos, além de outros maiores, que correspondem a grânulos de lipofuscina (Epstein, 1995).

O estroma prostático é complexo, apresentando células musculares esqueléticas e lisas, fibroblastos, nervos e células endoteliais.

Na porção mais distal da próstata, a musculatura esquelética do diafragma urogenital se estende para o tecido prostático, especialmente nas regiões periféricas (Kost & Evans, 1964; Manley Jr, 1966).

À exceção das células endoteliais, que formam os vasos e garantem o suprimento sangüíneo do órgão, as demais células do estroma têm função desconhecida, mesmo embora todas respondam à regulação androgênica, por apresentarem maior ou menor quantidade de receptores de andrógenos (Prins et al. 1991; El-Alfy et al. 1999)

Normalmente, as células musculares lisas são esparsas, cruzando-se de forma frouxa, geralmente ao redor das unidades glandulares, na maior parte do tecido prostático. Na região de transição, entretanto, elas são compactas e forma feixes grossos. Juntamente com as células musculares do esfíncter da base da bexiga, elas atuam no controle do fluxo urinário. A região de transição é o sítio de maior ocorrência de hiperplasia prostática benigna (McNeal, 1978), o que, na maioria dos casos, leva a obstrução urinária e distúrbios miccionais.

As células musculares lisas são responsáveis, a princípio, pela contração do órgão na ejaculação, fazendo com que a secreção prostática acumulada nos ácinos seja eliminada. Atualmente, sabe-se que estas células têm um papel preponderante nos mecanismos de estimulação parácrina, especialmente sobre o epitélio (Farnsworth, 1999), mas provavelmente também atuam sobre os próprios fibroblastos e demais células estromais. As células musculares lisas parecem ser o principal alvo da regulação α-adrenérgica da fisiologia prostática (Smith et al. 1999).

Interações epitélio-estroma

Interações entre o epitélio e o mesênquima são importantes em diversos estágios da morfogênese, na diferenciação celular e na função geral dos epitélios. Em vários sistemas foi demonstrada a ação de células mesenquimais/estromais e/ou de participação de vários

componentes da matriz extracelular na diferenciação celular e na manutenção do estado diferenciado (Hall, 1978; Hay, 1981; Hay, 1983; Trelstad, 1987; Lin & Bissel, 1993).

No caso da próstata, a função secretora do epitélio é regulada por andrógenos, que têm participação direta na diferenciação e na manutenção do estado ativo da glândula (Cunha et al. 1985; Donjacour & Cunha, 1993). Por outro lado, a morfogênese do epitélio parece sofrer influência indireta de andrógenos, pois tanto o sino urogenital como os brotos prostáticos não apresentam receptores para andrógenos em níveis detectáveis, enquanto o mesênquima do sino urogenital e da próstata em formação apresenta grande quantidade destes receptores. Isto sugere que a ação dos andrógenos na morfogênese prostática deve ser indireta, atuando via mesênquima, uma vez que parte significativa do crescimento epitelial e da ramificação dos ductos, que é dependente do estímulo androgênico, ocorre na fase em que as células epiteliais não apresentam receptores para andrógenos (Donjacour & Cunha, 1988).

Sabe-se que os fibroblastos têm ação no estímulo parácrino de origem mesenquimal sobre o epitélio prostático (Kabalin et al. 1989). Também já foi demonstrada a ação de diferentes fatores de crescimento, isolados ou em combinações, sendo produzidos por células estromais prostáticas (Story et al. 1989; Sutkowski et al. 1992; Kassen et al. 1996), que são capazes de estímulos autócrinos e, sem dúvida, de atuarem sobre o epitélio. Uma densa rede de interrelações entre diferentes fatores de crescimento foi proposta (Davies & Eaton, 1991).

Grande importância tem sido atribuída às células musculares lisas, tanto no controle homeostático da próstata, quanto no desenvolvimento neoplásico (Cunha et al. 1996, Farnsworth, 1999).

Foi também demonstrado um arranjo diferenciado das células do estroma prostático nas diferentes porções dos lóbulos prostáticos, tendo sido evidenciada uma maior quantidade de fibroblastos e poucas células musculares lisas nas porções terminais dos lóbulos, que estão associadas ao crescimento da população de células epiteliais. Por outro lado, uma pequena quantidade de fibroblastos associada a uma maior quantidade de células musculares lisas foi também observada nas porções proximais, que estão associadas à morte celular epitelial. Estes dados revelam que os arranjos assumidos pelas células estromais na próstata podem estar relacionados com a fisiologia das células epiteliais (Nemeth & Lee, 1996).

Considerando a possível ação de outros fatores solúveis sobre o desenvolvimento prostático, resta também a possibilidade de atuação da matriz extracelular na ligação de fatores de crescimento concentrando-os em microambientes específicos, reduzindo sua susceptibilidade à proteólise e aumentando sua afinidade aos receptores celulares de superfície (Roberts et al. 1988; Massagué, 1991). Obviamente, isto não se aos hormônios androgênicos.

Na regressão prostática causada pela castração, há uma significativa diminuição do tamanho e peso da glândula. Este quadro origina-se de uma parada na síntese dos produtos de secreção e aumento na velocidade de eliminação dos produtos glandulares, da perda de células epiteliais por apoptose e de mecanismos de autofagocitose, resultando em lóbulos menores formados por um epitélio cúbico baixo (Brandes, 1966; Kerr & Searle, 1973; Brändström et al. 1994). As modificações ultraestruturais envolvendo a regressão epitelial têm sido bem estudadas (Brandes, 1966).

Durante a regressão prostática, existe um aparente aumento na área seccional ocupada por matriz extracelular e células estromais (Kerr & Searle, 1973). Dentre estas últimas, as mais proeminentes são as células musculares lisas que se encontram ao redor das estruturas epiteliais (lóbulos e ductos). Elas assumem um fenótipo mais sintético, com uma fração miofibrilar reduzida (Zhao et al. 1994) e participam na remodelação da matriz extracelular (Vilamaior, Taboga & Carvalho, manuscrito em preparação).

Células estromais respondem a fatores de crescimento e influenciam as células epiteliais em experimento de co-cultura (Kassen et al. 1996) e, pelo menos na presença de fatores adicionais, as células musculares lisas são capazes de responder ao estímulo pela dihidroxitestosterona (Kassen et al. 1996).

Em trabalho anterior, pudemos verificar que as células musculares lisas são excluídas das áreas de proliferação glandular, dissociam-se das de mesmo tipo e ficam isoladas e progressivamente atróficas quando as células epiteliais tumorais invadem o estroma (Siviero & Carvalho, 1999). Com base neste trabalho anterior, pareceu-nos que uma investigação à microscopia eletrônica de alguns destes aspectos relationados às células musculares lisas seria importante.

Objetivo

O objetivo deste trabalho foi identificar aspectos ultra-estruturais das células musculares lisas durante a proliferação glandular e após a invasão epitelial em tumores da próstata humana.

Smooth muscle cell behavior in human carcinomas: Phenotypical variation at the ultrastructural level

Eduardo Scortegagna[1], Sebastião R. Taboga[2], Hernandes F. Carvalho[1]

[1]Dept. Cell Biology – UNICAMP, CP6109, 13083-970 Campinas SP and [2]Dept. of Biology – IBILCE – UNESP, 15054-000 São José do Rio Preto SP, Brazil

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Correspondence and reprints addressed to:

Hernandes F. Carvalho
Dept. Cell Biology – UNICAMP
CP6109
13083-970 Campinas SP, Brazil
Tel./fax: 0055 19 788 7821
E-mail: hern@unicamp.br

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Abstract. The ultrastructural aspects of the prostatic smooth muscle cells during glandular proliferation and epithelial invasion in selected tumor cases are presented. We report that smooth muscle cells react upon epithelial proliferation by loosing the homotypic contacts and accumulating extracellular matrix components in the intercellular spaces. Upon invasion, the smooth muscle cells show either progressive degeneration or atrophy. The degenerated phenotype was characterized by nuclear collapse and expansion of the perinuclear space, cytoplasmic vacuolization and detachment from the disrupted basal lamina, while atrophic cells showed a diminished cytoplasmic to nuclear ratio and reduction and disruption of the basement membrane. An "activated" phenotype was also found and refers to smooth muscle cells that show extensive surface folding and association with extracellular matrix components, in special collagen fibrils and elastin, and peripheral cytoskeletal disorganization and accumulation of vesicular material. The conversion between these different phenotypes is possible and an acceptable sequence is proposed. These phenotypical modifications are likely attributed to either the lack of paracrine stimulation (given the transformation of epithelial cells) or to the degradation of the basal lamina of the smooth muscle cells by tumor-derived proteases.

Introduction

A growing body of experimental evidences implies the smooth muscle cells (SMC) on the control of prostatic epithelial cell structure and function. In the rat prostate, an organized distribution of smooth muscle cells, more concentrated in the proximal regions of the tubulo-acinar secretory units was suggested to be responsible for a less proliferative state and a propensity for apoptosis in the normal organ, in contrast to the distal parts, in which the main stromal cells are fibroblasts and the epithelial behavior is more proliferative (Nemeth & Lee, 1996). This pattern was also associated with an increased production of TGF-beta by stromal cells in the rat ventral prostate (Nemeth et al. 1997). A plethora of humoral factors mediating stroma-epithelium interactions in the normal prostate does exist (Davies & Eaton, 1991; Farnsworth, 1999). Recently, autocrine and paracrine interactions were demonstrated to be mediated by FGF9, which was shown to be produced by SMC and be mitogenic for SMC and epithelial cells (Giri et al., 1999).

It has also been suggested that stromal cells influence epithelial prostate cancer growth to varying extent in different stages of tumor establishment and progression (Condon & Bosland, 1999) and that the tumor neovascularization is due in part to a stromal reaction (Farnsworth, 1999).

Cunha et al. (1996) presented evidences for a balance between normal epithelial cell and smooth muscle cell in the prostatic homeostasis and that normal epithelial cells support SMC differentiation, while transformed or tumoral epithelial cell are unable to induce SMC differentiation. It allowed those authors to propose that paracrine interactions between these two cell types are lost during neoplastic development.

The fate of the prostatic SMC following epithelial cell invasion of the prostatic stroma is unknown. They were reported to be activated in benign prostatic hyperplasia, showing a striking increase in the volume fraction of secretory organelles (Bartsch et al. 1979; Brueenger et al. 1983). Indeed, the predominance of fibroblasts in neoplasms may indicate that SMC undergo a dedifferentiation program and adopts a more fibroblastic phenotype, accounting at least in part to the functions attributed to "activated fibroblasts" in the tumoral tissue (Tremblay, 1979; van den Hooff, 1981).

However, a study on the structural modifications underwent by SMC during epithelial proliferation and invasion of the stroma is still lacking. A previous study, at the light microscope level, showed that SMC become progressively isolated and atrophic with carcinoma installation and progression (Siviero & Carvalho, 1999)Thus, we decided to investigate the features of the SMC in some selected prostatic tumors (graded according to Gleason's score), aiming at establishing some aspects of their fate, as epithelial cells progressively invade the stroma and become metastatic. The present work presents our ultrastructural findings on the SMC reaction and identify different SMC phenotypes likely related to the stromal reaction to epithelial proliferation.

Material and methods

Material: Specimens were obtained from four patients (56-73-year-old) subjected to radical prostatectomy in the Passo Fundo University Hospital, Passo Fundo, Rio Grande do Sul State, Brazil.

Transmission electron microscopy: Tissue fragments from the different prostatic zones (transition, central peripheral, anterior fibromuscular stroma) were fixed by immersion in a solution of 3% glutaraldehyde and 0.25% tannic acid in Millonig's buffer (Cotta-Pereira et al. 1976) for 4-24hs. Material was then washed with buffer and post-fixed in 1% osmium tetroxide in the same buffer for 1h. After additional washes, the material was dehydrated in graded acetone and embedded in Epon 812 resin. Thin sections were obtained and stained with toluidine blue. Areas of interest, revealing different stages of tumor progression, considering structural aspects described by Gleason (1977), were selected and further sectioned for transmission electron microscopy. Silver sections were stained with uranyl acetate and lead citrate and observed in a Leo 906 transmission electron microscopy opperating at 60-80kV.

Results

The smooth muscle cells (SMC) of non-affected areas of the human prostate present close contact with each other, showing homotypic contacts in the formation of bundles (Fig. 1). They are large, spindle shaped cells which share a common basement membrane (BM) with the adjacent neighbor either laterally or in an end-to-end fashion (Fig. 2), this intimate association is also revealed by the observation of complementary outlines, when seen in cross-section (Fig. 3). The SMC are not necessarily grouped, and some of them are isolated showing no lateral association with other SMC. The nuclei are large and elongated, with peripheral accumulation of clumped chromatin, and the nucleoli are prominent (Figs. 1-4). In normal cells the cytoskeletal contractile components are tightly apposed the cell nucleus (Fig. 4). In some areas, the BM is apparently expanded (Fig. 2). SMC bundles are separated by extracellular matrix (Figs. 1-3), composed mainly of collagen fibrils, associated in fibers and elastic fibers. Some SMC showed areas devoid of cytoskeleton (apparently an empty vacuole) and dense or residual bodies (Figs. 1-3).

In the areas adjacent to proliferating but not invasive carcinoma, the smooth muscle cells are isolated in the matrix. This isolation seems to occur by the progressive accumulation of collagen fibrils and, likely other extracellular matrix components between the SMC. Fig. 5 shows a cell with prominent folding and/or projection of cell processes, which are in most of the cases associated with collagen fibers or elastin. These aspects are associated with the peripheral accumulation of large vesicles and with a disorganization of the cytoskeleton in these areas (Figs. 5 and 6). Figs. 7 and 8 are other aspects of the SMC represented by an increase in the proportion of the synthetic organelles to the cytoskeletal component, as compared to the normal cells. The cytoskeleton is restricted in these cases to some microfibril bundles in the cytoplasm. The identification of these cells as SMC is based in the presence of pinocytotic vesicles, dense plaques under the cell membrane and the BM.

Some SMC showed an extensive accumulation of the peripheral vacuoles (Figs. 9 and 10) and a disruption of the cytoskeleton, revealed by the existence of large components filled with an amorphous substance with moderate electron density. In some SMC the connections

between the marginal dense plaques and the plasma membrane were lost and the space was filled with amorphous substance (Fig. 10).

In the areas of epithelial cancer cells invasion, SMC were mostly atrophic (Figs. 11-14). The atrophy was observed as a decreased ratio of the nuclear to cytoplasmic area, resulted from the loss of the cytoskeletal components. Atrophy apparently followed cell isolation by the extracellular matrix deposition or epithelial cancer cell invasion. Close proximity (Figs. 11 and 12) and even intimate association between the two cell types were observed. In every case, the atrophic cells had diminished and interrupted BM.

Some smooth muscle cells showed marked aspects of degeneration, represented by extensive expansions of the perinuclear spaces, loss of the cytoskeleton, a decrease in connection between the cytoskeleton and the plasma membrane, and ruptures of the cellular integrity (Figs. 15-17). In some areas, complex associations with other cell types were observed (Fig. 18). In these cases, the BM was redundant and the cellular limits were difficult to define.

Some cells located in the stroma showed an increased synthetic activity, as indicated by the amount of RER and the enlargement of its cisternaes (Fig. 20), as compared to the usual fibroblasts (Fig. 19). The former showed enlarged RER cisterns and Golgi.

Discussion

The results presented herein define some aspects of the SMC in the stroma in cases of prostate carcinomas. The SMC show a generalized set of ultrastructural modifications associated with the progressive epithelial proliferation and stromal invasion.

In the low grade carcinoma, there seems to exist an initial reaction of the smooth muscle cell, represented by a gradual isolation of cells, which loose the contacts with the adjacent neighbors by the progressive accumulation of extracellular matrix, mainly represented by collagen and elastin.

We have shown before that in the areas of less differentiated tumor cells, corresponding to Gleason's score 5, the SMC are mostly atrophic (Siviero & Carvalho, 1999).

In this work, we could identify different phenotypes that correspond to the atrophic cells observed before. The atrophic cells themselves constitutes the first phenotype. The second one is an "activated" phenotype, apparently involved with extracellular matrix reorganization, as suggested by the intimate interactions with collagen and elastin. The third, "degenerated" phenotype is distinct from the other two, essentially by the nuclear collapse, cytoplasmic reduction and disruption of the BM.

Fig. 21 summarizes these observations and suggests some possible conversions between the SMC phenotypes. A logic sequence is that the activated cells become atrophic and then degenerated. However, it is possible that SMC adopt each of the three forms directly. It seems obvious that the degenerated phenotype proceeds to cell death and elimination.

It has been observed in the prostatic stroma of rats, that the SMC respond differently to diverse physiological situations. Castration, for instance, results in the death of some SMC, but in activation of others, which play an important role in the stromal remodeling attained by androgen depletion (Vilamaior, Taboga and Carvalho, manuscript in preparation).

In the present study, we observed progressive changes in the SMC in relation to the tumor establishment and progression. It is apparent that the SMC lack the usual cellular organization and interactions and that at least some of them degenerated. Whether these degenerative aspects correspond to apoptotic events specific to the SMC remains to be determined. Another important aspect is the possible adoption of a fibroblastic phenotype.

Many authors assume that fibroblasts are activated and produces a series of tumor specific components, in special angiogenic factors that are essential for tumor progression (Folkman, 1995; Franck Lissbrant et al. 1997; Farnsworth, 1999). Cunha et al. (1996) have also suggested that the stromal reaction observed in neoplasia, and frequently attributed to fibroblasts, may have a contribution from the SMC. Fibroblasts were also observed in our preparations and they showed different content and organization of the secretory organelles. The relationships between "normal" and "activated" fibroblasts and the "dedifferentiated" SMC could not be ascertained by the present study, and remain elusive.

Epithelial invasion seems to involve disruption of the epithelial BM (Flug and Köpf-Maier, 1995). It was demonstrated that cancer cells produce a large amount of BM

components, without forming an evident structure, likely by the simultaneous production of proteolytic enzymes (Bonkhoff, 1998). This enhanced turnover of BM components demonstrate that they are important for these cells to immigrate into the surrounding interstitial stroma (Bonkhoff, 1996; 1998). We have observed progressive loss of the BM structure in the different SMC phenotypes. It is possible that proteolytic enzymes produced by the tumor cells act on the BM of the SMC and that disruption of this structure contributes to the phenotypical changes of these cells. The BM disruption may also be a causative agent in addition to a deficient signaling from the epithelial compartment (Cunha et al. 1996), since BM is important in keeping the differentiated phenotype of many cell types (Leblond & Inoue, 1989; Flug & Köpf-Maier, 1995).

From the results obtained here we may suggest that the SMC reaction to neoplasia follow initial steps (isolation and atrophy) but then is variable resulting in the different phenotypes observed. It is also possible that these different phenotypes result from different SMC populations in response to the lack of proper stimulation from the epithelium and from the disruption of the BM.

Finally, it is possible that the moderate accumulation of peripheral vesicles and the presence of dense bodies in the smooth muscle cells may represent aging effects. To confirm this hypothesis, a comparison with younger and healthy samples is needed.

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Figure legends

Fig.1. Low power view of the prostatic stroma in a non-affected area. A bundle of SMC is observed. Cells are closely associated with each other and the BM is fused between two adjacent cells. The cytoplasm is filled with contractile elements of the cytoskeleton, interrupted only by some large vesicles that are observed in some of the SMC (arrowheads). A dense (or residual) body is pointed by the arrow. Collagen fibrils are found in the spaces between the SMC bundles. X4,175.

Fig. 2. Smooth muscle cells of the prostatic stroma in a non-affected area, showing expanded BM between adjacent cells (stars), corresponded to the fusion of BM from each of them. It takes place laterally and in a end-to-end fashion (arrows). The cell nucleus is observed in one of the SMC and shows a prominent nucleolus. X 4,527.

Fig. 3. Connections between adjacent SMC are represented by the fusion of the BM (arrows) and by the complementary surface outlines between adjacent cells. Large vesicles are present at the cell periphery (arrowhead). X 6,430.

Fig. 4. Some SMC are not laterally associated with other cells, but involved by the extracellular matrix. The SMC in this figure is long and the cell nucleus is also elongated. Ep=epithelium. X 6,960.

Fig. 5. A smooth muscle cell close to an area of glandular proliferation. The cell shows areas of cytoskeletal disorganization (long arrows) in which there is an accumulation of peripheral vesicles (short arrows) and many projections of the cell surface that intimately associate with collagen and/or elastin fibers (arrowheads). The cell nucleus is slightly clumped and the perinuclear space is slightly expanded. X 4,630.

Fig. 6. A SMC in an equivalent area as in fig. 5. The cell shows a marked disorganization of the surface with numerous infoldings and residual dense plaques (arrowheads). Large vesicles (short arrows) are accumulated in an lateral expansion of the cytoplasm (long arrows). X 6,960.

Fig. 7. A SMC with a reduced cytoplasm with a predominance of synthetic organelles. The BM and the peripheral vesicle, typical of the SMC are still seen (arrowheads). An adjacent cell shows extensive cytoplasmic vacuolization (arrows). X 14,400.

Fig. 8. A SMC with reduced cytoskeletal components, restricted to some bundles (asterisks) and residual dense plaques. The cell nucleus has loose chromatin and a prominent nucleolus. X 14,870.

Fig. 9. Part of a SMC showing aspects related to the accumulation of peripheral large vesicles (arrowheads), a dense body (short arrow) and a large disruption of the cytoskeleton, filled with an amorphous material (long arrow). Ep=epithelial cell; col=collagen fibrils. X 8,570.

Fig. 10. Adjacent SMC with accumulation of peripheral large vesicles (arrowheads). One of the SMC shows a retraction of the cytoskeletal components and residual dense plaques from the plasma membrane and the BM, resulting in a large area filled with frocculent material (double headed arrow). X 8,630.

Fig. 11. Atrophic SMC, in the proximity of a invading epithelial cancer cell (Ep). The cytoplasmic area is reduced and the cell nucleus show an irregular outline. Mitochondria are grouped . Large peripheral vesicles are seen in the cytoplasm of the SMC (arrowheads). The spaces between the SMC is widened by the accumulation of collagen fibrils (arrows). X 13,670.

Figs. 12-14. Atrophic SMC of the prostatic stroma, in areas of epithelial cancer cell (Ep) invasion. The cells show clumped nuclei and expanded perinuclear spaces. The cytoplasm is

scant. In figure 14 an epithelial cancer cell is in intimate association with a SMC. Disruption of the BM is frequent (arrows in fig 12). The arrow in fig. 13 points to a large expansion of the perinuclear space and the arrowheads show the peripheral vesicles common to the SMC. In figure 14, the arrowheads show disruptions of the BM. Fig. 12, X5,110; Fig. 13, X2,325; Fig. 14, X21,000

Fig. 15. A degenerated SMC in close proximity to a epithelial cancer cell (Ep), showing extensive expansion of the perinuclear space, disruption of the cell periphery (arrow). X14,400.

Fig. 16. A degenerated SMC, with expanded perinuclear spaces (arrows) and compacted cell nucleus. The cytoplasm is reduced. X6,500.

Fig. 17. Another degenerated SMC with expanded perinuclear space (stars), reduced cytoplasm and residual dense plaques (arrows). The cell is in close proximity to epithelial cancer cells (Ep). X 11,615.

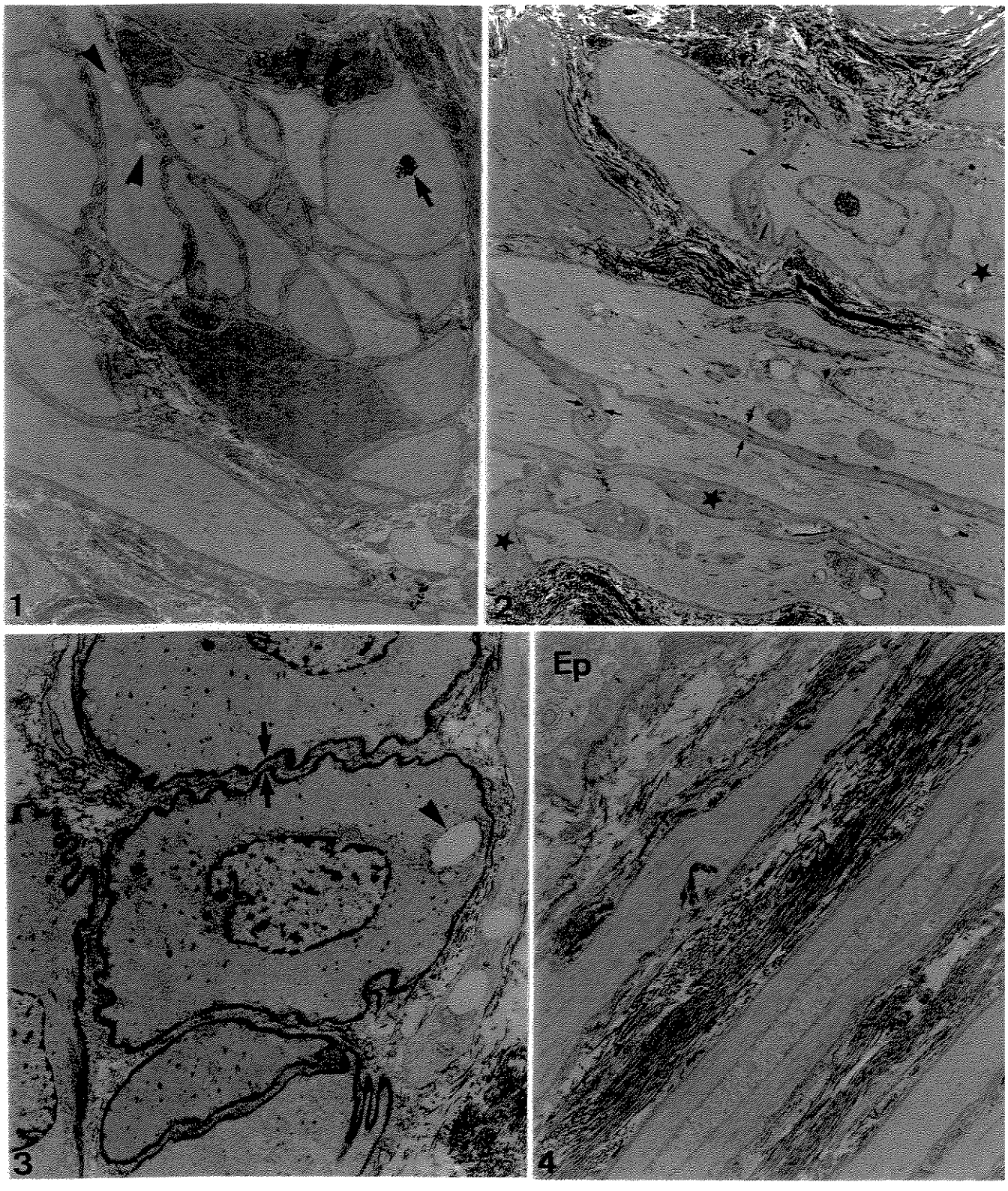
Fig. 18. Degenerated SMC are grouped in an stromal area close to invading epithelial cancer cells. Redundant (asterisk) and disrupted (arrows) BM are observed. Areas of cytoplasmic retraction, filled with an amorphous material, are marked by stars. X5,965.

Fig. 19. A typical fibroblast (Fb) of the prostatic stroma, showing accumulation of rough endoplasmic reticulum and a prominent nucleolus in a large cell nucleus. X 8,270.

Fig. 20. An atypical fibroblast of the prostatic stroma, with enlarged cisterns of the RER (stars). Whether it represents a dedifferentiated SMC or an activated fibroblast is unknown. X8,990.

Fig. 21. Schematic drawing of the SMC phenotypes observed in the prostate stroma in cases of prostate carcinoma. The prostatic SMC is a spindle-shaped cell with elongated nuclei with

condensed chromatin associated with the inner part of the nuclear envelope. The atrophic phenotype is characterized by a diminished cytoplasmic to nuclear area ratio, revealing the loss of the contractile component. The cell nucleus is clumped and the perinuclear space is enlarged in the areas of nuclear retraction. The BM is unusually thin and less conspicuous than in the normal cells. The activated phenotype corresponds to cells having an increased number of synthetic organelles and/or showing extensive infolding of the cell surface, usually associated with residual dense plaques and cytoskeletal disorganization in the peripheral areas. Association with fibrillar components of the extracellular matrix suggests an active role in tissue reorganization. The degenerated phenotype shows condensed and collapsed nuclei with expanded perinuclear space and extremely reduced cytoplasm. The BM is almost completely lost and organelles are scant. The SMC may give rise to each of these phenotypes directly. However, a transition from the activated state, through the atrophic one to the degenerated situation is possible. The dedifferentiated cell can not be distinguished from fibroblast at the ultrastructural level, but this phenotype may exist. The arrows indicate possible direct phenotypical changes, while the interrupted arrows indicate putative indirect conversions. The dedifferentiated phenotype could not be recognized.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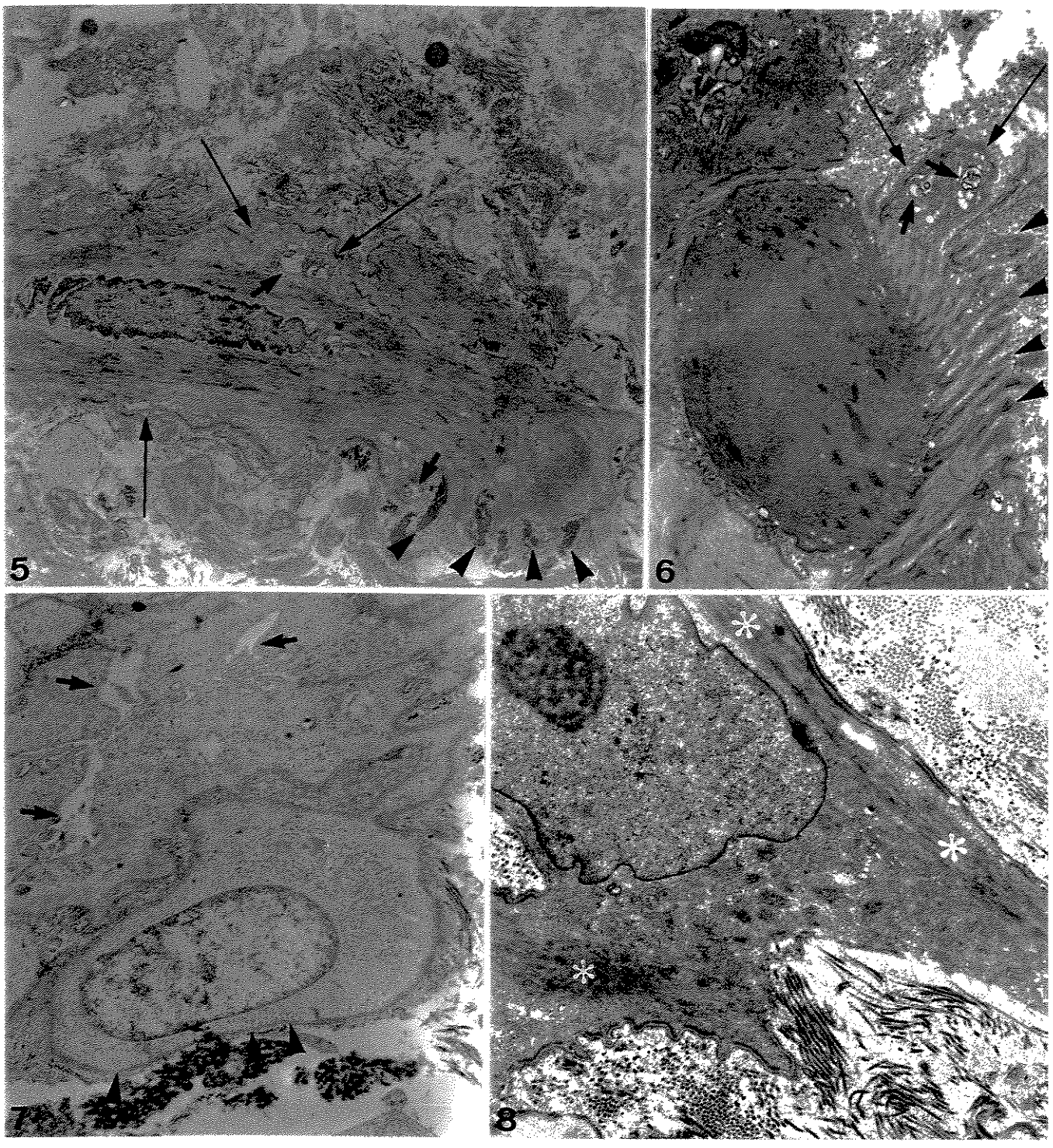
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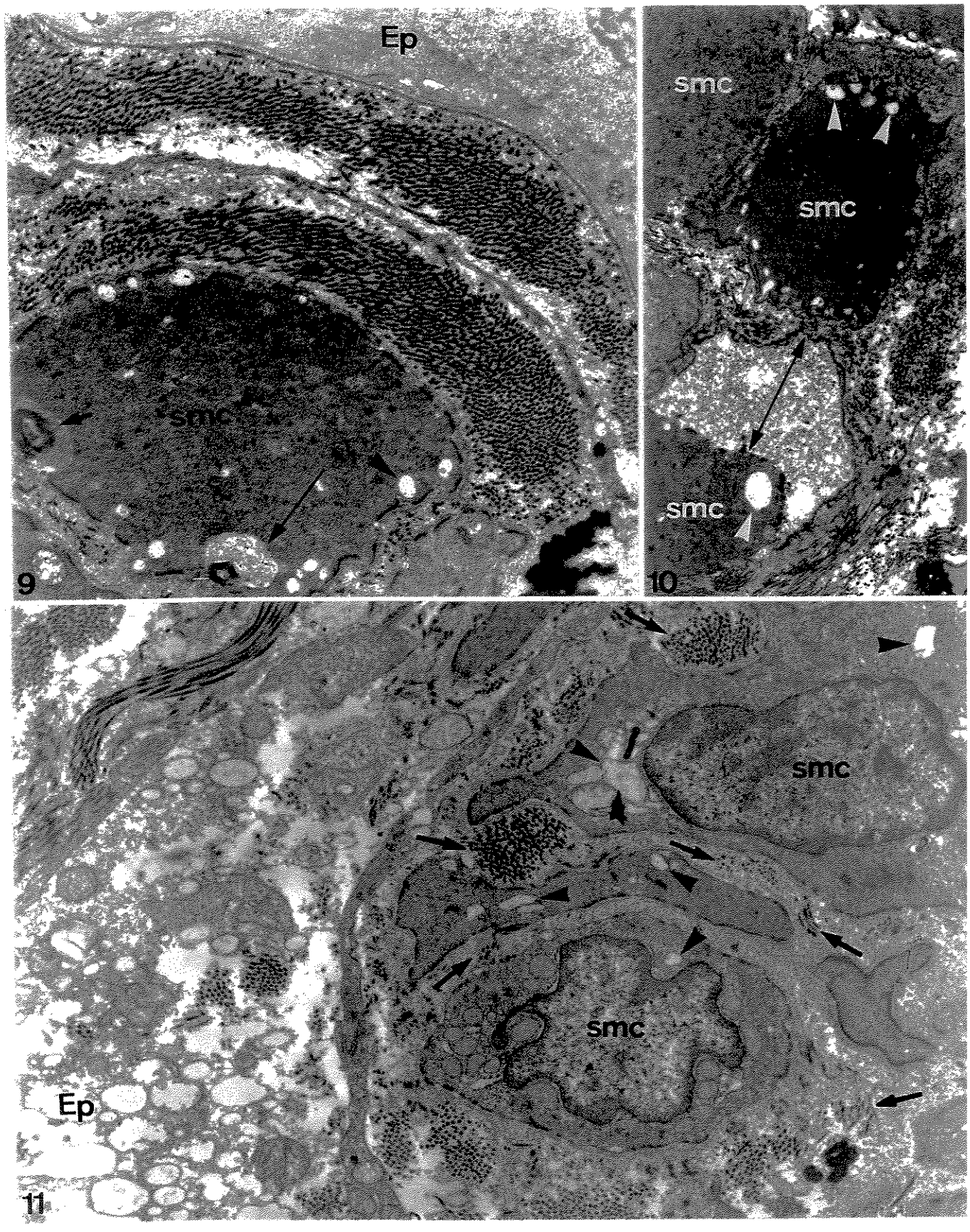
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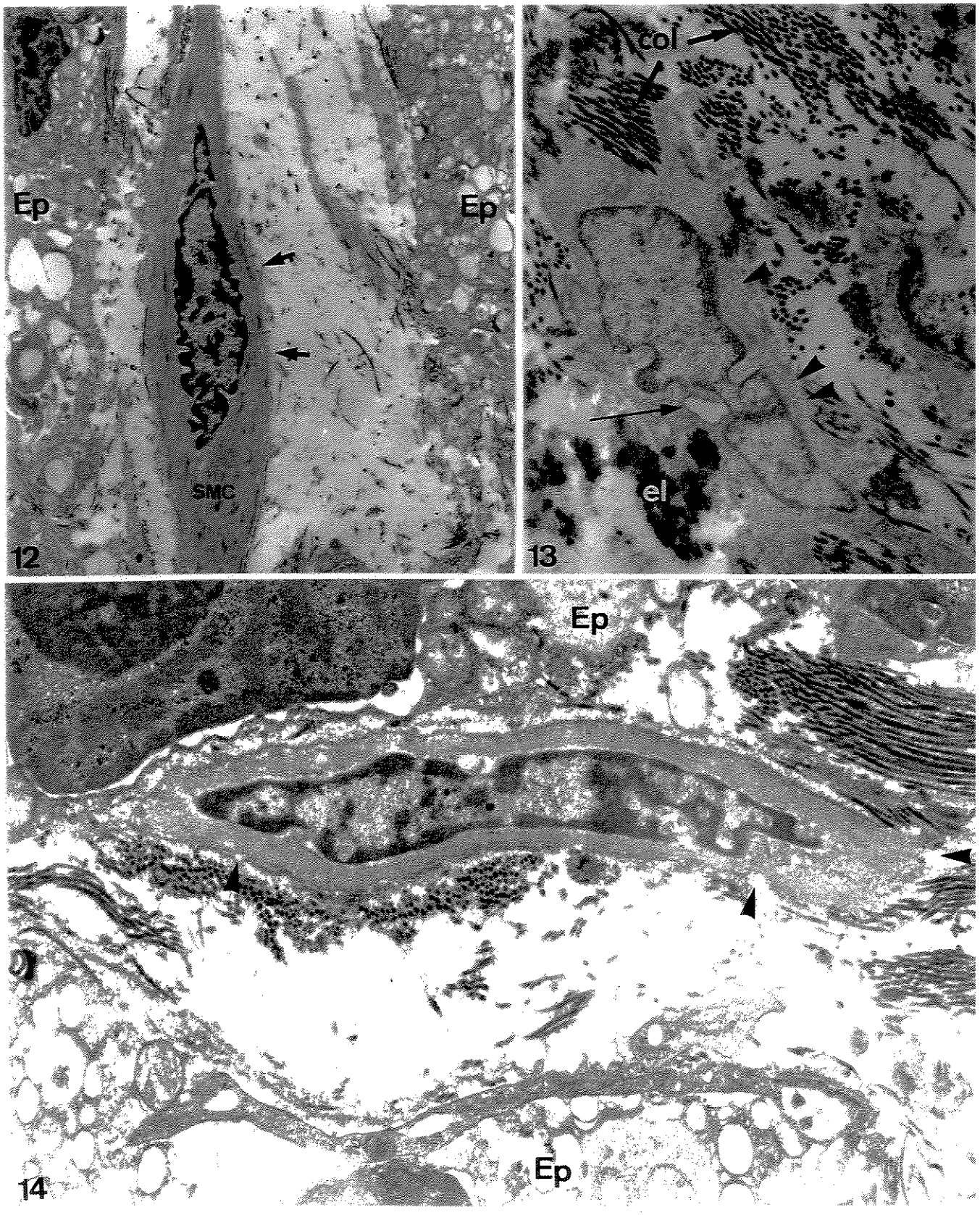
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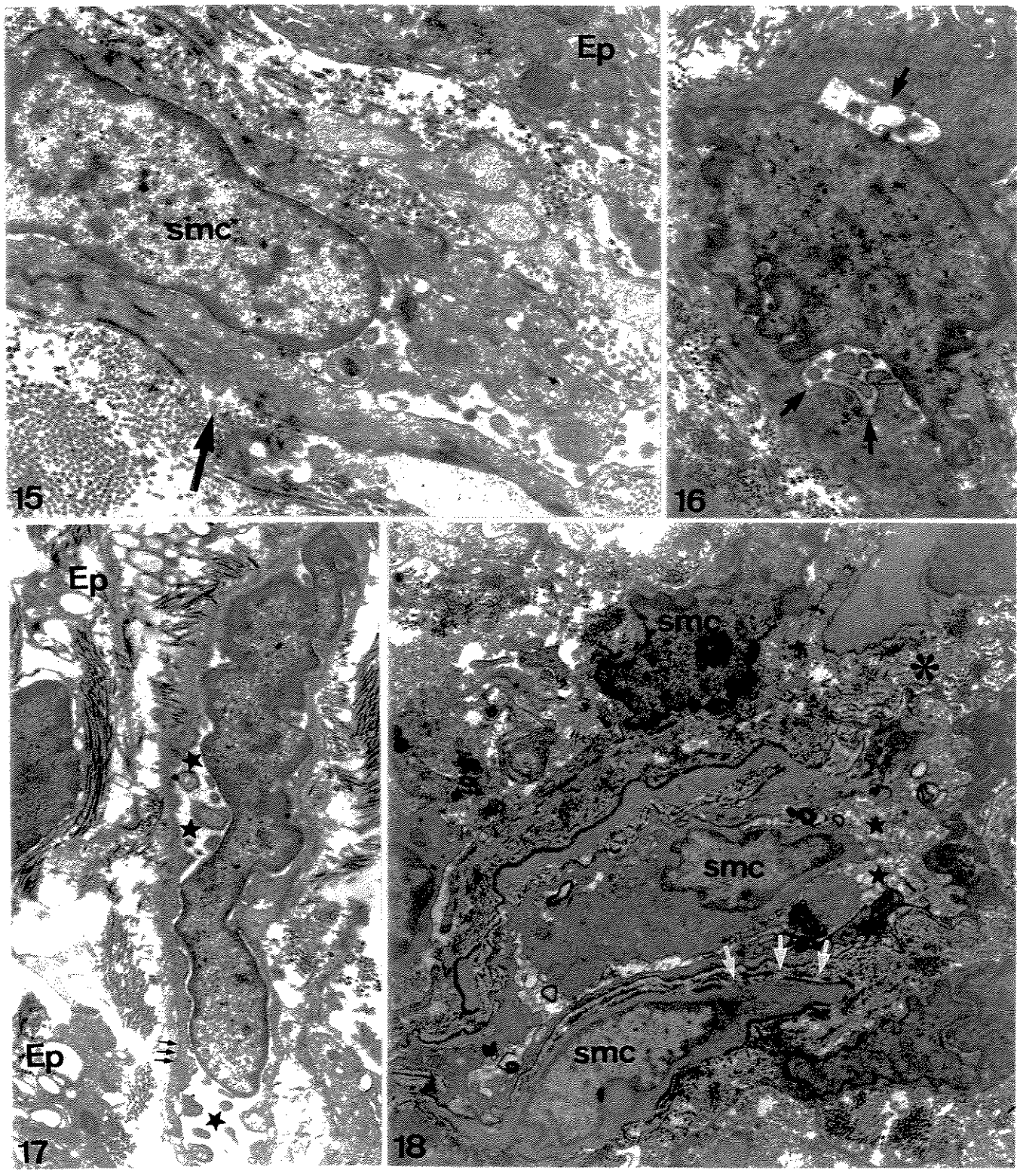
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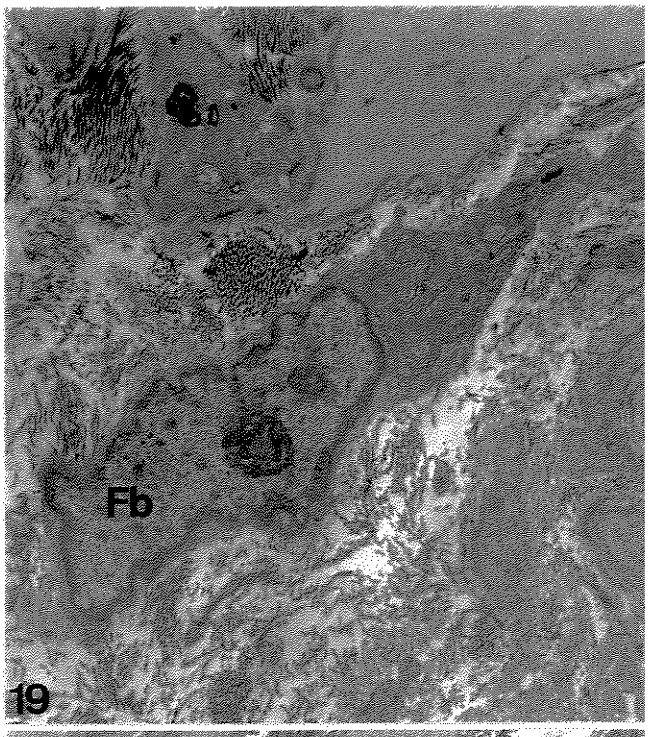
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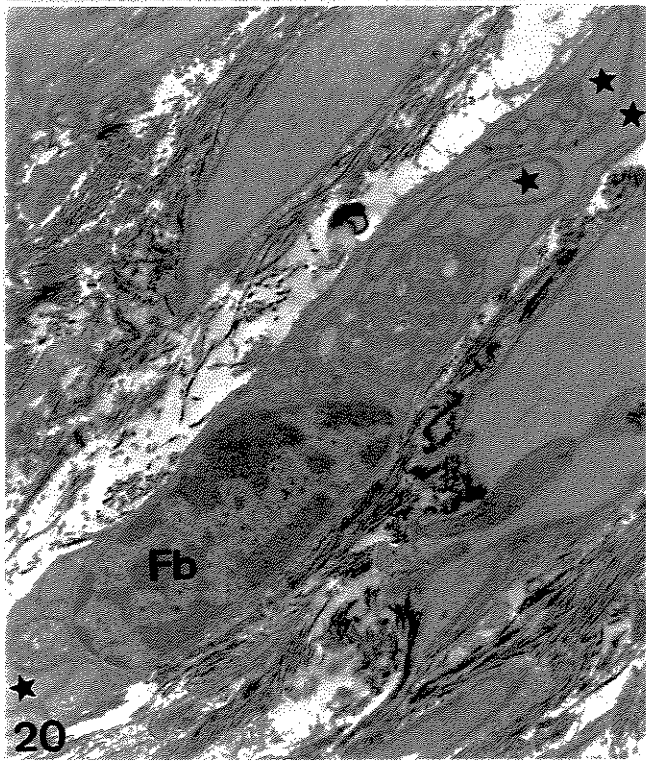
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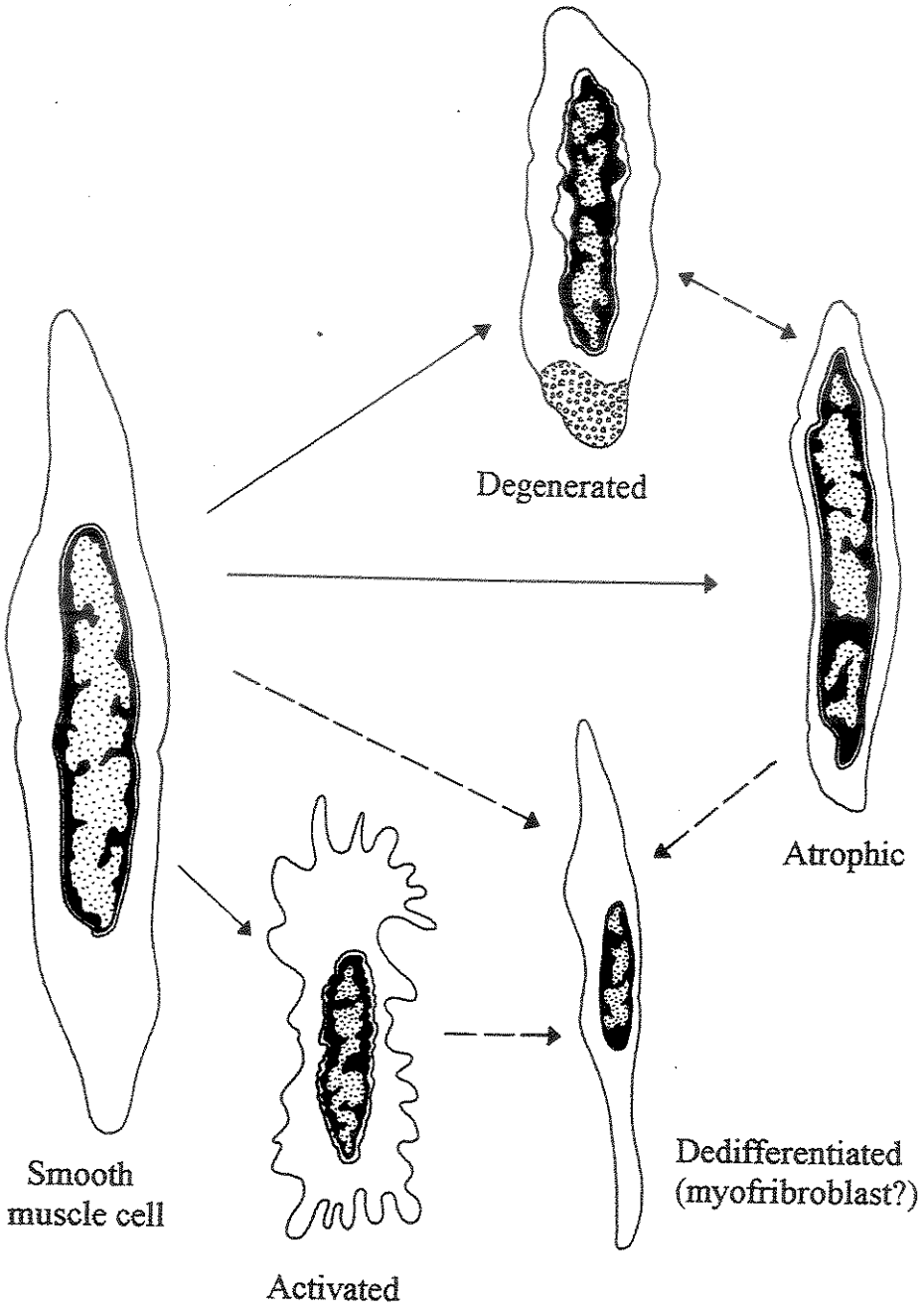
Degenerated
Atrophic
Smooth
muscle cell
Activated
Dedifferentiated
(myofribroblast?)

21

Conclusões gerais

1. As células musculares lisas do estroma prostático modificam-se fenotipicamente frente à proliferação glandular e invasão tumoral

2. Existem alterações iniciais, caracterizadas pela perda de contato entre as células musculares lisas e pelo isolamento das mesmas pelo acúmulo de componentes da matriz extracelular.

3. As células musculares lisas tornam-se predominantemente atróficas quando da invasão epitelial, mas podem assumir outros fenótipos, caracterizados aqui como *ativado* e *degenerado*.

4. Transições entre os diferentes fenótipos parecem existir e o fenótipo degenerado provavelmente antecede a morte e eliminação celular.

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