Mammary gland development and cancer

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Summary

The mammary gland is a complex organ that begins development early in gestation and constantly changes in size, shape and function from the time of puberty to menopause. The earliest stages of embryogenesis appear to be independent of steroid hormones, whereas after the 15th week breast structure is largely influenced by a variety of hormones. In most females, further breast development begins at puberty under the influence of cyclical estrogen and progesterone secretion. This process may continue into the 20s and it is enhanced by pregnancy. Growth and transcription factors contribute to the reciprocal stromal-epithelial interactions in growth, development and tumorogenesis of the mammary gland. From the embryological point of view the morphology of both mammary ductal and lobular cells results from the same developmental process. Numerous data suggest the existence of self-renewing, pluripotent mammary stem cells but their molecular characteristics and differentiation pathways are unknown. The extensive research currently being done in molecular biology and pathology, cancer genomics and proteomics will hopefully contribute to further elucidation of all the genetic and environmental factors involved in the development, differentiation, and involution of the mammary gland and this may give insight into the etiopathogenesis, early detection, treatment, and potential prevention of breast cancer.

Key words: **mammary gland development – differentiation – growth factors – transcription factors – ductal and lobular cells – breast cancer**

Souhrn
Prsní žláza – vývoj a nádory

Prsní žláza je komplexní orgán, jehož vývoj začíná záhy v gestaci, a který se od puberty k menopauze průběžně mění ve velikosti, tvaru i funkci. Zatímco nejčasnější stadia embryogeneze probíhají nezávisle na steroidních hormonech, po 15. týdnu je struktura prsu významně ovlivněna řadou hormonů. U většiny žen začíná po pubertě další vývoj prsu pod vlivem cyklické sekrece estrogenu a progesteronu. Tento proces může pokračovat i po dvacítce a je potencován těhotenstvím. Růstové a transkripční faktory přispívají ke vzájemným interakcím stroma – epitel při růstu vývoji normální prsní žlázy, i při patogenezi. Z pohledu embryologie vychází morfologie duktálních i lobulárních buněk prsu z téhož vývojového procesu. Existují četné údaje o existenci obnovujících se pluripotentních kmenových buněk prsní žlázy, avšak jejich molekulární charakteristika a cesty diferenciace nejsou dosud známy. Lze doufat, že probíhající rozsáhlý výzkum molekulární biologie, patologie a nádorové genomiky a proteomiky přispěje k objasnění všech genetických i zevních faktorů účastnících se vývoje, diferenciace a involuce prsní žlázy, a zároveň povede k lepšímu pochopení etiopatogeneze, časnému záchytu, léčbě a možná i prevenci karcinomu prsu.

Klíčová slova: **prsní žláza – vývoj – diferenciace – růstové faktory – transkripční faktory – duktální a lobulární buňky – karcinom prsu**

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Mammary gland embryogenesis

Over the past few years, significant new insights have been made in our understanding of the transcription factors that guide development of the mammary gland. The human mammary glands develop from the mammary ridges which first appear in 5-week fetus. The ridge does not develop further and disappears during fetal development (22, 54). On the chest wall, mesenchymal condensation occurs around an epithelial stalk at about the 15th week. Further

development of the buds into ductal structures involves reciprocal signaling between the epithelium, the mammary mesenchyme and the stromal fat pad (56). Inhibins and activins regulate mammary epithelial cell differentiation through mesenchymal-epithelial interactions (53). Growth of cords of epithelium into the mesenchyme produces a group of solid epithelial columns, each of which give rise to a lobe in the mammary gland. The papillary layer of the fetal dermis gives rise to vascularized fibrous tissue (40). Coincidentally, differentiation of the mesenchyme into fat within the last 2 months of gestation and canalization of the epithelial cords occur, followed by development of branching lobuloalveolar glandular structures. Near birth, the nipple is formed. Between birth and puberty the mammary gland is relatively quiescent, with only limited ductal elongation into the mammary fat pad (22, 23).

LeBrun et al. found that the expression of bcl-2 protein is more widespread in fetal than adult tissues, which is consistent with its role in promoting cell survival, presumably by preventing apoptosis (16, 33, 38). Immunohistochemical localization of Bcl-2 has been detected in the basal epithelium of the developing breast bud and in the surrounding stroma. Similar patterns of staining have been reported in male and female tissue. Bcl-2 is not present in the epithelium of the normal adult breast. These observations suggest that up-regulation of bcl-2 contributes to morphogenesis of the fetal breast by its inhibitory effect on apoptosis (44).

Postnatal development and hormonal regulation of mammary gland

The earliest stages of fetal mammary gland development appear not to be dependent on steroid hormones, whereas the actual development of the breast structure after the 15th week is largely influenced by testosterone (22). In the last weeks of gestation, the fetal breast is responsive to steroid hormones and prolactin, which is manifested after birth by the secretion of colostrum and palpable enlargement of the breast bud. The secretory activity typically subsides and ceases during the first or second month after birth (23, 26).

The morphological changes that the mammary gland undergoes during puberty, pregnancy and lactation are primarily defined by steroid and peptide hormones (42). In most females, further breast development does not begin until puberty (70). With the onset of cyclical estrogen and progesterone secretion at puberty, adolescent breast development commences and involves rapid ductal outgrowth and branching (53). Branching emanates from the terminal end buds, which are specialised regions occuring at the tips of the growing ducts. These contain the precursor cells to the luminal epithelial and myoepithelial cells. Growth of ducts and differentiation of periductal stroma occur at this time in estrogen-dependent manner (2, 69). Growth hormone and glucocorticoids contribute to ductal growth. Lobuloalveolar differentiation and growth during this period are enhanced primarily by insulin, progesterone, and growth hormone. By the end of puberty, a simple ductal system or primary and secondary ducts are developed (58, 59).

Anbazhagan et al. devised a system of classification to study the extent of the structural development of the ductal system (morphological types I, II, and III) and the functional differentiation of the lining epithelium (functional stages I to V) in infant breasts collected at necropsy. There was no correlation between the age of the infant and the type of development of the ductal system. There were no distinguishing features between breasts from the two sexes. Myoepithelial cells were present at all stages and prominent staining for casein was observed up to 2 months of age (1).

Breast glandular differentiation may continue into the 20s and it is enhanced by pregnancy. During pregnancy, further ductal side-branching occurs together with lobuloalveolar proliferation leading to the formation of the lobuloalveolar compartment with secretory epithelium. This phase of development is driven by progesterone, prolactin and other pregnancy-induced hormones (43, 45). Stat5 (signal transducer and activator of transcription) pathway is critical for the determination, proliferation, and differentiation of mammary alveoli during pregnancy (25). Geymayer and Doppler provide evidence for a role of NF-kappaB (nuclear factor kappa-B) in normal mammary gland development, and indicate its function as a negative regulator of beta-casein gene expression during pregnancy by interfering with STAT5 tyrosine phosphorylation (19). Miyoshi et al. demonstrated that signalling via the PrlR and Stat5 is critical for the proliferation and differentiation of mammary alveoli during pregnancy (41). The observations of Philp et al. revealed a complex pattern of activation of STAT factors during mammary growth, differentiation and remodelling and provided the evidence for the involvement of STAT3 in development of the mammary gland (49). It has been shown that during pregnancy beta-casein synthesis is induced in single mammary epithelial cells via an integrin-dependent pathway (27, 53). Beta1-

integrins play an important role in the control of milk gene transcription and in the maintenance of the mammary epithelial cell differentiated state (15).

SRC-1 (steroid receptor coactivator-1) family members interact with steroid receptors, including ERa (estrogen receptor alpha) and PR (progesterone receptor), to enhance ligand-dependent transcription. Shim et al. documented that SRC-1 is not necessary for ERa-mediated induction of PR in mammary epithelial cells and is also not sufficient for PR induction in stromal cells expressing both ERa and SRC-1 (63). Chughtai et al. report that Src homology 2 (SH2) domain containing protein-tyrosine phosphatase SHP-2 contributes to prolactin receptor (PRLR) signal transduction to beta-casein gene promoter activation. These studies indicate a tight physical and functional interaction between SHP2 and Stat5 required for PRL-mediated signalling in mammary cells (7). It has been shown that p21-activated kinase (Pak) 1, a serine/threonine protein kinase, is activated in mammary glands during pregnancy and lactation (72). Following weaning, the mammary gland undergoes extensive remodeling through apoptosis of the secretory cells, returning the gland to a virgin-like state (22, 59).

Saji et al. found that ERa and ERb are expressed in the rat mammary gland but the presence and cellular distribution of the two receptors are distinct. In prepubertal rats, ERa was detected in 40 % of the epithelial cell nuclei. This decreased to 30 % at puberty and continued to decrease throughout pregnancy to a low of 5 % at day 14. Cells coexpressing ERa and ERb were rare during pregnancy, a proliferative phase, but they represented up to 60% of the epithelial cells during lactation, a postproliferative phase (60). Forster et al. suggest a role for ERb in organization and adhesion of epithelial cells and hence for differentiated tissue morphology. Because a reduced risk for breast cancer is conferred on women who breast-feed at an early age, ERb could contribute to this risk reduction by facilitating terminal differentiation of the mammary gland (18). Russo et al. concluded that the content of ERa and PR in the normal mammary tissue varies with the degree of lobular development, in parallel with cell proliferation. However, the expression of receptors occurs in cells other than the proliferating cells, indicating that they represent at least two separate cell populations (57). Shyamala et al. found that PR present in a heterogeneous manner in breast epithelial cells are essential for lobuloalveolar and not for ductal morphogenesis. Progesterone signalling through PR may occur through paracrine mechanism and regulated expression of the two isoforms of PR is critical for maintaining appropriate responsiveness to progesterone and epithelial cell replicative homeostasis (64).

Growth factors in mammary development and carcinogenesis

Growth factors contribute to the reciprocal stromal-epithelial interactions in growth, development and tumorogenesis of the mammary gland. Signalling by members of the epidermal growth factor receptor family plays an important role in breast development and breast cancer. It has been shown that EGFR (epidermal growth factor receptor) is essential for mammary ductal growth and branching morphogenesis, but not for mammary lobulo-alveolar development (73, 74). Cripto-1 (CR-1) also known as teratocarcinoma-derived growth factor-1 is a novel EGF-related protein that induces branching morphogenesis in mammary epithelial cells and inhibits the expression of various milk proteins. CR-1 is also overexpressed in mammary tumors (62).

The ERBB (receptor protein-tyrosine kinase) family of type 1 receptor tyrosine kinases and their ligands play important role during mammary morphogenesis. ERBB4 functions as an essential mediator of the mammary differentiation factor STAT5 signalling, and that loss of STAT5 activity contributes to the impaired functional differentiation of mammary glands observed in mice containing conditional Erbb4 deletions (37). Jones et al. found that ErbB4 signalling is necessary for terminal differentiation of mouse mammary gland and Stat5 activation at mid-lactation (28). Kazansky et al. detected immunocytochemically considerable cytoplasmic and some nuclear staining for Stat5a1 during late pregnancy and predominantly nuclear staining during early lactation (29). Yamashita et al. proposed that serine phosphorylation within the transactivation domain may limit the activity of Stat5a in the absence of proper coactivation by glucocorticoid receptors. Costimulation of glucocorticoid receptors completely reverses the suppressive effect of Stat5a serine phosphorylation on beta-casein gene transcription (76).

Osin et al. found that the distribution of growth factors and extracellular matrix proteins play a significant role in different cellular compartments during morphogenesis of the developing human breast. TGF-alpha (transformating growth factor) immunoreactivity was observed both in the stromal and the epithelial cells within fetal and infant breasts up to 25 days. TGF-beta 1 immunoreactivity was localized in the extracellular matrix (46). In addition, tenascin-C

was found around the neck of the developing breast bud and in the extracellular matrix of the infant with peaks in the newborn at 6–12 weeks (61).

Keratinocyte growth factor/fibroblast growth factor 7 (KGF/FGF7) is known to be a potent growth factor for mammary cells but its origin, cellular targets and mode of action in the breast are unclear. Palmieri et al. showed epithelial and myoepithelial immunohistochemical staining for FGF7 in normal breast sections, and epithelial staining in breast carcinomas. FGF7 stimulated proliferation of both epithelial cell types, but not stromal fibroblasts. Interleukin-1beta caused a dose-related FGF7 release (47).

It has been shown that TGF-beta 1 is located in the mammary gland periductal and intraductal stroma, closely associated with epithelial or myoepithelial cells in both benign and malignant breasts (3). The relative localization of these two growth factors in the mammary gland may be significant in the control of breast development and/or tumor formation and progression (20).

Recent studies have documented that PTHrP (parathyroid hormone-related protein) is a native product of mammary epithelial and myoepithelial cells and is necessary for mammary development (17). In the absence of a functional PTHrP gene, mammary development fails during embryonic life, and no mammary epithelial ducts are formed. This molecule participates in the regulation of epithelial-mesenchymal interactions not only during embryonic mammary development, but also during adolescent ductal morphogenesis. In addition, PTHrP plays a critical role in the establishment of bone metastases in breast cancer (13). The recent experiments have demonstrated that PTHrP and the PTH/PTHrP receptor represent an epithelial/mesenchymal signalling circuit that is necessary for mammary morphogenesis and stromal cells are a critical target for PTHrP's action in the mammary gland (14, 75).

Transcription factors in mammary gland development

The Ets (expressed sequence tags) gene family consists of 27 genes that encode sequence-specific transcription factors. Genetic analyses suggest roles for Ets proteins in embryonic development and various adult physiological processes (39). The overexpression of some ETs genes is linked with numerous malignancies, including breast cancer. The PEA3 (transcription factor polyomavirus enhancer activator protein) subfamily proteins play key regulatory roles in both mammary gland development and oncogenesis (32). The bHLH (basic helix-loop-helix) family of transcription factors functions in the coordinated regulation of gene expression, cell lineage commitment, and cell differentiation in most mammalian tissues. Id proteins function as dominant negative regulators of bHLH transcription factors (9). Lin et al. identified Id-1 protein is a critical regulator of these normal mammary epithelial cell phenotypes, also regulator of aggressive and invasive phenotype and mediator of the effects of sex steroid hormones, in human breast cancer cells (36).

C/EBPalpha and -delta, the members of C/EBP (CCAAT/enhancer binding protein) family of bZIP transcription factors, are also expressed in mammary gland. The multiple protein isoforms of C/EBPbeta play a critical role in mammary gland development and cancer. Targeted deletion of all the C/EBPbeta isoforms results in a severe inhibition of lobuloalveolar development and a block to functional differentiation and ductal morphogenesis (21). Qi et al. found that nuclear receptor coactivator peroxisome proliferator-activated receptor-interacting protein (PRIP) is important for normal mammary gland development (50).

Mammary gland development is characterized by dynamic changes in the expression and functions of protein kinases. Perturbations in the regulated expression or function of protein kinases or their associated signalling pathways can lead to malignant transformation of the breast. Since receptor-tyrosine kinases regulate several essential processes such as mitogenesis, motility, invasion, cell survival, and angiogenesis, targeting receptor-tyrosine kinases may have important implications in designing strategies against breast cancer (31). Recent genetic and expression analyses strongly suggest that homeobox genes may perform similar functions at specific developmental transition points in the mammary gland. These analyses also suggest that homeobox genes may play a contributory or causal role in breast cancer (34).

To date, six main pathways affecting mammary morphogenesis have been described. These include: (1) LEF1 (lymphocyte enhancer factor 1) and b-catenin signalling, (2) homeobox genes Msx1, Msx2 and Hox signalling (5), (3) steroid hormone receptor signalling, (4) STAT signalling, (5) Helix-loop-helix (HLH) gene signalling and (6) c-myc, Ets and LMO4 oncogene signalling (71). All can also be involved in breast cancer development (fig.1).

The data above discussed show that from the embryological point of view there exists no substantial difference between mammary ductal and lobular cells and that the morphology of both

results from the same developmental process. There is no evidence for a different gene expressing profile in normal ductal and lobular cells. However, some investigations report differential immunohistochemical markers of ductal and lobular cells.

Differential immunohistochemical markers of ductal and lobular cells

An essential feature of mammary gland biology is its ability to regenerate a functional epithelium upon successive cycles of lactation and involution. Determination of the cellular pathways through which this recapitulation of organ growth and differentiation occurs will provide an important framework for mammary gland conceptualizing mammary dysplasia and carcinogenesis (67). Numerous data suggest the existence of self-renewing, pluripotent mammary stem cells (8, 10); however, their molecular characteristics and differentiation pathways are largely unknown (30, 55). Transformed mammary stem or progenitor cells undergo aberrant differentiation processes that result in generation of the phenotypic heterogeneity found in human and rodent breast cancers (12).

It is suggested that epithelial/intermediate stem cells exist in a basal position predominantly in terminal structures of growing breasts. The acquisition of the malignant phenotype is associated with the carcinoma cells having a greatly impaired ability to differentiate to myoepithelial and to alveolar cells (55). Pechoux et al. and Deugnier et al. suppose that human mammary luminal epithelial cells contain progenitors to myoepithelial cells (48, 11). Stingl et al. reported the evidence for the existence of multilineage HBEC (normal mammary epithelial cell line) progenitors in normal adult human mammary tissue. The MUC-1+/CALLA-/ESA+ and the MUC-1- to +/-/CALLA +/- to +/ESA+ progenitors are candidates in vivo alveolar and ductal progenitors, respectively (68).

Recent transgenic mouse models have demonstrated that distinct pre-committed mammary epithelial progenitors function to regenerate mammary ducts and secretory mammary lobules. Lobular, i.e. secretory progenitor cells are present as entities among the mammary epithelial cells found in immature virgin female mice. Similarly, ductal epithelial progenitors are present within the same popu-lation. Lobular progenitors are present in greater numbers, although both cell populations are ex-tremely small. The results may indicate coope-rative interaction between the two epithelial progenitors or signal the presence of a third progenitor type, i.e. a mammary epithelial stem cell capable of producing both ductal and lobular-committed daughters (6, 35).

The study of Hebbard et al. suggests that the CD44v6 epitope is expressed in mammary epithelial stems cells and in lineages derived from these cells, and that CD44v6 expression is regulated in part by hormones and growth factors such as IGF-1 (insulin-like growth factor 1) and EGF (epidermal growth factor) which regulate the growth and differentiation of the mammary epithelium. The expression of the CD44v6 epitope observed in some mammary tumors reflects the stem cell origin of breast tumors (24).

14-3-3sigma is a candidate tumour suppressor gene trans-activated by p53 in response to DNA damage. 14-3-3sigma is preferentially expressed by myoepithelial cells. Simpson et al. demonstrated that 14-3-3sigma was consistently expressed in the cytoplasmic compartment and occasionally in the nuclei of myoepithelial cells arranged as a continuous layer around normal ducts and lobular units. 14-3-3sigma cytoplasmic subcellular localization was a statistically significant prognostic factor for the whole series of invasive carcinomas, as well as for those positive for ER or PR (65).

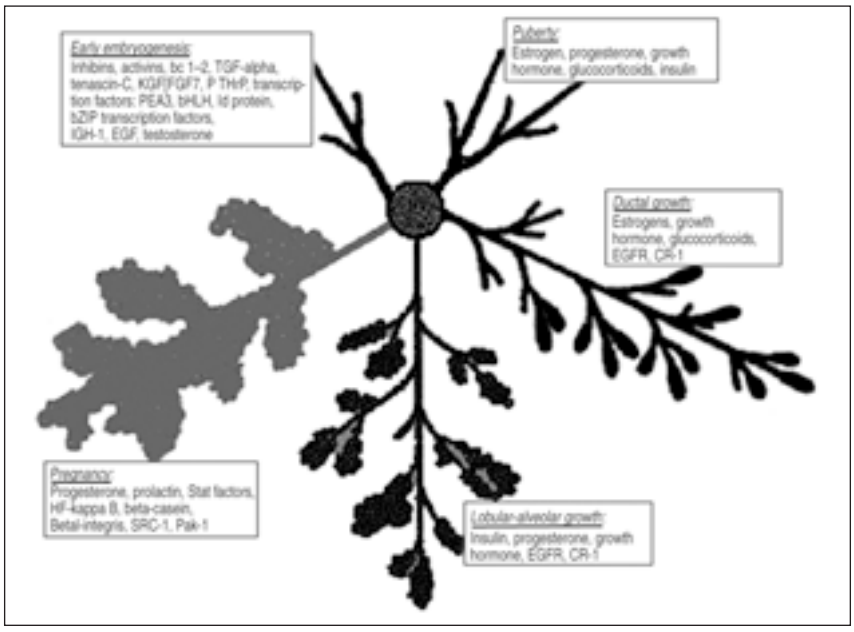

Fig. 1. Schematic representation of mammary gland development

Using an antibody against the P2X7 (apoptotic purinergic receptor), Slater et al. found that normal and mildly hyperplastic epithelium was devoid of the cytolytic P2X7 receptors whereas all epithelial cells in all cases of in situ or invasive lobular or ductal carcinoma labelled intensely. The lobular and ductal in situ cases labelled intracellularly while the invasive epithelial cancer cells showed intense cell surface label indicating an attempt was being made to induce apoptosis (66).

The study of Barsky confirmed the hypothesis that human myoepithelial cells express a distinct tumor-suppressor phenotype. The global gene expression profile (22,000 genes) was examined using Affymetrix Microarray Gene Chips. The myoepithelial cell lines/ xenografts were distinct and very different from nonmyoepithelial breast carcinoma cells and normal breast and breast tumor biopsies. Two hundred and seven specifically selected genes represented a subset of genes that distinguished all the myoepithelial cell lines/xenografts from all the other samples and which themselves exhibited hierarchical clustering (4).

Conclusion

The mammary gland is a complex organ that begins development early in gestation and constantly changes in size, shape and function from the time of puberty to the menopause, affected by varying levels of the female hormone estrogen. All those changes are associated with a variety of hormonal and genetic risk factors and subsequent development of breast cancer. From the embryological point of view there exists no substantial difference between mammary ductal and lobular cells and the morphology of both results from the same developmental process. There is no evidence for a different gene expressing profile in normal ductal and lobular cells. The extensive research currently being performed on the molecular pathology, molecular biology, cancer genomics and proteomics will hopefully contribute to the further elucidation of all genetic and environmental factors involved in the development, differentiation, and involution of the mammary gland. This may give insights into the etiology, pathogenesis, early detection, treatment, and potential prevention of breast cancer.

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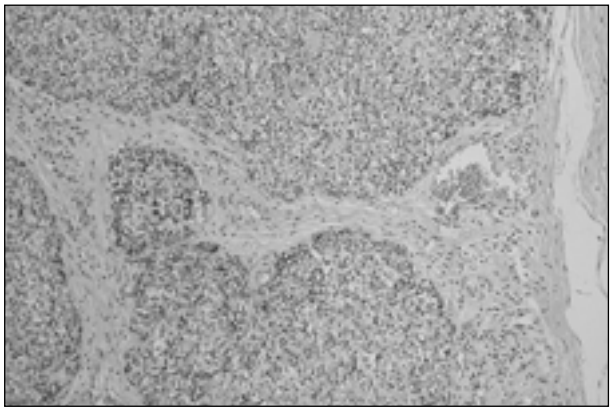
Jaká je vaše diagnóza?

73letá žena s devět měsíců trvajícím nebolestivým zduřením v pravé parotideomaseterické krajině. Při fyzikálním vyšetření byl zjištěn v této lokalizaci kulovitý útvar průměru 40 mm s intaktním kožním krytem. Poruchy funkce příušní slinné žlázy nebyly přítomny, inervace n. VII byla zachována. Ultrasonograficky byl v příušní slinné žláze zastižen hyperechogenní útvar rozměrů 27 x 16 mm a bylo doporučeno jeho odstranění. Při operaci byl zjištěn nádor v povrchovém laloku žlázy, který utlačoval, ale neprorůstal větvení n. VII. Byla provedena pouze exstirpace nádoru, po které následovala aktinoterapie. Devět měsíců po operaci a šest měsíců po poslední kůře aktinoterapie je pacientka bez klinických známek lokální recidivy či generalizace nádorového onemocnění.

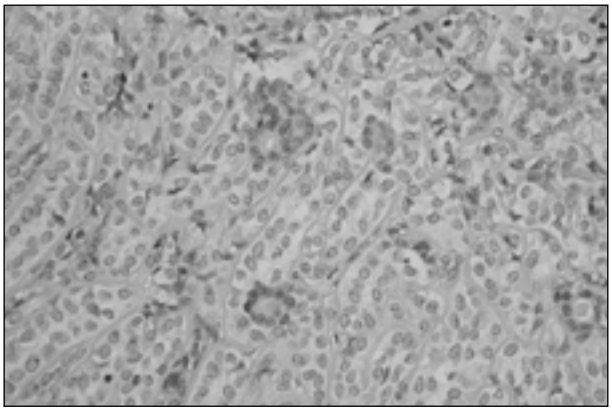
K histologickému vyšetření byla zaslána kulovitá částice 35 x 30 x 20 mm s dobře ohraničeným uzlem tužší konzistence průměru 20 mm. Na řezu byl uzel homogenní, šedobílé barvy.

Mikroskopicky byl zastižen vazivově opouzdřený, solidně rostoucí nádor, subkapsulárně lobulárně uspořádaný (obr. 1), který vykazoval v zásadě bifazickou strukturu. Většinu nádorové populace tvořily velké polygonální buňky se světlou cytoplazmou, které se nacházely periferně od drobných lumin vystlaných kubickými buňkami s eozinofilní cytoplazmou (obr. 2). V některých luminech byl přítomen eozinofilní materiál. Stroma nádoru bylo chudé. Nádorové elementy nevykazovaly výraznější jaderné ani cytologické atypie, mitózy byly řídké (2–3 na 10 HPF).

Imunohistochemicky duktální epitelie exprimovaly silně cytokeratiny (CK) a nekonstantně a slabě epiteliální membránový antigen (EMA) a S-100 protein. Světlobuněčná komponenta nádoru naopak silně exprimovala hladkosvalový aktin (SMA), protein p63 a S-100 protein; vimentin byl exprimován pouze fokálně. Stroma nádoru bylo silně PAS pozitivní, stejně jako materiál v drobných luminech. Světlobuněčná komponenta nádoru byla PAS negativní. Stroma nádoru, stejně jako materiál v drobných luminech, vykazovalo též pozitivitu při stříbření dle Jonese. Výsledek barvení na hlen byl negativní, stejně jako imunohistochemický průkaz GFAP. Proliferační antigen Ki-67 byl exprimován v nečetných buňkách, a to výhradně v buňkách se světlou cytoplazmou (do 5 %), převážně v periferních, resp. subkapsulárních partiích nádoru.



Obr. 1. Solidně rostoucí, ložiskově lobulárně uspořádaný nádor je obdán vazivovým pouzdrem, z něhož vycházejí do nádorové tkáně neúplná septa (HE, 100krát)



Obr. 2. Bifazické uspořádání nádoru (HE, 400krát)

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