Relevant infrastructural alterations in invasive pancreatic ductal adenocarcinoma

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Abstract

In this study, we focus our interest on some peculiar infrastructural abnormalities detected in a pancreatic cancer case. Our electron microscopic observations underline the high plasticity of the pancreatic parenchyma cells. Tumor pancreatic exocrine lesions are represented by putative ductal and acinar cells, which proliferate and grow in a haphazard pattern, detrimental to endocrine counterpart. The tumor cells do not exhibit neither a pure ductular or ductal nor a pure acinar phenotype, but tumor lesions represented by neoplastic ductal cells with invasive growth are by far prevalently. In our pancreatic cancer case, electron microscopic investigation clearly shows that a plethora of the epithelial cells from the tumor lesions contain large areas of autophagy leading to the pleomorphic inclusions represented by fibrillar/filamentous inclusions frequently associated with hyaline-amorphous material, and secondary lysosomes. One of the most striking and important finding in this report for a case of pancreatic cancer is the high fragility (extensive dissolutions) of plasma membrane of tumor cells leading to pseudo-syncytia formation. Desmosomal junctions are severely altered, almost missing. Plasma membranes showed shedding membrane vesicles. Extravasated inflammatory cells contribute to the dramatic and extensive destructive areas of epithelial cells as well as tumor-stroma counterpart, including the basement membrane. All above severe infrastructural abnormalities, especially down regulation of cell-cell and cell-extracellular matrix adhesions might result in aberrant cell behavior and, consequently, much care should be taken for the postoperative patient evolution.

Keywords: pancreatic ductal adenocarcinoma, shedding membrane vesicles, plasma membrane fragility, pseudo-syncytia.

Introduction

Neoplasms of the pancreas can be divided into (1) neoplasms with predominantly exocrine differentiation and (2) neoplasms with predominantly endocrine differentiation. Mostly of pancreatic malignant tumors involve exocrine pancreas. Pancreatic ductal adenocarcinoma (PDAC, nomenclature derives from its histological resemblance to ductal cells) is the predominant tumor in the pancreas (85–90% of all the pancreatic tumors), and is usually referred to as pancreatic cancer (PC) [1, 2]. Endocrine component of the pancreas can be also involved in developing tumors, so-called pancreatic neuroendocrine tumors (PNETs), but mention must be made that almost are benign tumors. Less than 3% of primary pancreatic neoplasms result from neuroendocrine tumors [3].

PC is one of the most aggressive human solid tumors, with a very rapid rate of growth and metastatic spread, a high resistance to chemotherapeutic drugs, and one of the highest fatality rates of all cancers. PC is a common cause of death among solid cancers, with 40 000 estimated deaths/year in Europe and approximately 30 000 deaths/year in the USA [4, 5]. PC affected frequently males than females, blacks than whites. According to the American Cancer Society, pancreatic cancer (PC) is considered as the fourth leading cause of cancer death, with the majority (approximately 80% of cases) occurring in people 60–80 years of age or older in western countries. It rarely develops before the age of 40–50 years [6, 7]. When the diagnosis of unresectable disease is made, about 80% of patients with pancreatic adenocarcinoma cannot benefit of curative strategy, but some palliative actions can be applied [8].

PC occurs most commonly in the head of pancreas. PC occurs twice as frequently in the pancreatic head (70% of cases) as in the body (20%) or tail (the least common location of 10%) of the gland [7]. When located in head pancreas, pancreatic carcinoma usually presents with obstructive jaundice, while tail pancreatic carcinoma tend to appear later and larger than pancreatic head carcinoma. Moreover, tail pancreatic carcinoma exhibits signs of advanced disease, such as contiguous organ extension, vascular invasion and distant metastases [9]. Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers due to the difficulty in establishing early diagnosis and ineffective chemo and radiation therapies. The tumor metastasis can arise in a wide variety of organs, but mostly in regional lymph nodes, duodenum, liver, and peritoneum. The brain, lungs, kidneys and bones can be also ectopic places to host secondary PDAC tumors [2]. Mention must be made that not all pancreatic cancers are metastatic; 12% of patients had no metastatic disease at autopsy [10].

So far, three distinct PDAC precursors were identified: (1) pancreatic intraepithelial neoplasia (PanIN), (2)
mucinous cystic neoplasm and (3) intraductal tubulopapillary mucinous neoplasm \[11, 12\]. PanINs are the best characterized.

The transformation of normal duct epithelial cells into invasive adenocarcinoma takes place gradually through the formation of lesions of different morphological grades, consequently initiating diverse changes in the pancreatic histoarchitecture and functions of the cells (Figure 1).

Figure 1 – Diagrammatic representation of the pancreatic cancer progression from histologically normal epithelium through gradually increasing morphological abnormalities known as pancreatic intraepithelial neoplasia (PanIN): columnar ductal cells (PanIN-1A), low-grade dysplasia (PanIN-1B), moderate grade dysplasia (PanIN-2) to high-grade pancreatic intraepithelial neoplasia (PanIN-3), including invasive growing carcinoma inside of peritumoral stroma (TEM). Mention must be made that normal ductal epithelium expresses cilia while pancreatic intraepithelial dysplastic lesions and invasive tumor cells lost cilia. Moreover, a basement membrane (BM) is still preserved between the dysplastic epithelia and the adjacent stroma. BM is destroyed when neoplastic epithelium is growing invasive inside of the stroma. Atypical mitosis can be seen in PanIN3 and invasive carcinoma lesions (elliptic areas delimited by blue interrupted lines). Small red arrows mark shedding membrane vesicles generated by tumor cells. PMN: Polymorphonuclear cell; Fb: Fibroblasts; CAFb: Cancer associated fibroblasts; RBC: Red blood cell. (Adapted with modification from references [2] and [13]).

The precursor lesions of PDAC are termed as pancreatic intraepithelial neoplasia (PanIN) with a gradually evolution from PanIN-1A through PanIN-1B, PanIN-2, to PanIN-3 (in situ carcinoma). PanIN-1A appears as flat columnar ductal cells without important signs of cell abnormalities (atypia). PanIN-1B grade shows lesions with papillary architecture without atypia. Lesions with increased cell abnormalities and a prevalence of papillary architecture are characterized as either PanIN-2 (low to moderate grade of dysplasia) or PanIN-3 (high-grade dysplasia), the later is considered the stage immediately preceding stromal invasion. Basement membrane is destroyed when neoplastic epithelium is growing invasive inside of the stroma [2, 11, 13].

Little is known about the causes of pancreatic cancer. Cigarette smoking is the most consistent risk factor \[12\]. Patients with chronic pancreatitis or with long-standing diabetes mellitus are at increased risk. Obesity associated with sedentariness is also a risk factor for pancreatic cancer [7, 14].

Pancreatic cancer is fundamentally a genetic disease [1, 4, 13, 15, 16]. Like other solid tumors, pancreatic cancer is a result of the genetic alterations accumulation over many years leading to the genetic instability affecting oncogenes and tumor suppressor genes. Loss or gain of gene function may appear as activation (upregulation) of oncogenes, downregulation of genomic maintenance/DNA repair genes and housekeeping genes, as well as genes that control the apoptosis/cell death/immortalization cascade, upregulation of growth factors/growth factor receptor signalling cascade systems, alterations in cytokines and adhesion molecules expression [17]. It is well known that mutations in the K-RAS oncogene appear as one of the earliest genetic alterations detected in PDAC.
Relevant infrastructural alterations in invasive pancreatic ductal adenocarcinoma

being present in cca. 36% of PanIN-1A, growing progressively to 44% of PanIN-1B, 87% in PanIN-2/3 until 90% in PDAC [11]. Progressive genetic mutations in the KRAS2, TP53, SMAD4, c-MYC genes, which also are hallmarks of advanced PDAC, consistent with PanIN to PDAC progression model associated with PDAC have been detected in PanINs [1, 18]. Defective telomeres may be the major cause of the chromosomal instability observed in many cancers and in the vast majority of pancreatic cancers [15]. Loss of telomeric integrity within the epithelial duct cells is involved in PDAC development. Telomeres are repetitive TTTAGG nucleotides and some associated proteins among we mention TRF1 and TRF2 located at the end of the chromosomes arms that play an important role in stabilization of chromosome during cell division [19]. Approximately 90% of the lowest grade of PanIN lesions exhibits a marked shortening of the telomeres. Myc gene expression is deregulated in 15% to 30% of human cancers, resulting in elevated levels of the Myc protein [20, 21], which may lead to telomeric aggregate formation and consequently to the genomic instability [19].

Malignancy is usually associated with metastasis; before to metastasis, some specific cell behavior takes place in order to perform invasion of exocrine pancreatic malignant transformed cells. In this context, we may assume that transmission electron microscopy investigations may provide relevant information about PC development, diagnosis and prognosis. Here, we investigate by electron microscopy a case of pancreatic carcinoma, the examination suggesting that pancreatic neoplastic lesions are represented by putative ductal cells intermingled with presumptive acinar areas. We underline that, to some extent, some infrastructural abnormalities can be analyzed in order to accurately evaluate the type of morphologic lesions.

Materials and Methods

We report a case of PC in a 74-year-old female patient. At the time of referral to the Department of Surgery of “St. Pantelimon” Emergency Hospital, Bucharest, Romania, she had been diagnosed with progressive loss of weight (approximately 8 kg in the past three months), loss of appetite combined with fatigue and sclerotegumentary jaundice with some pruritus. The abdominal CT scan showed tumoral lesion in the head of the pancreas with no hepatic metastasis.

In order to perform transmission electron microscopy (TEM) investigations, small tissue fragments about 2–3 mm² from a normal pancreas, from the pancreatic tumor mass as well as from the peri-pancreatic tumor but uninvolved part of the pancreas (as control counterpart tissue) resulted by surgery as curative therapy for the patient suffering from pancreatic cancer (surgeon got patients’ consent) were processed following the routine transmission electron microscopy (TEM) protocol [22]. After pre-fixation in fresh ice-cold 4% glutaraldehyde in sodium cacodylate buffer, pH 7.4, for three hours, at 4°C, the tissues were six times washed in 0.05 M sodium cacodylate buffer (pH 7.4), at 4°C, postfixed in 2% osmium tetroxide in 0.1 M sodium cacodylate, at 4°C, for 2.5 hours, stained en-bloc with 0.5% aqueous uranyl acetate overnight, at 4°C, and washed with 0.05 M sodium cacodylate buffer, pH 7.4. After dehydration in graded series of ethanol and infiltration with propylene oxide, specimens were embedded in Glycid ether (Epon 812-equivalent) and finally polymerized at 60°C, for 48 hours. Semithin sections were stained with 1% toluidine blue for light microscopy. Ultrathin sections (80–100 nm) were cut using a diamond knife and collected on 200 mesh grids, and double counterstained with uranyl acetate and subsequently lead citrate. The ultrathin sections were examined in a JEOL JEM 1400 transmission electron microscope operated at an acceleration voltage of 80 kV. An Olympus video camera was used to perform images capture.

Results

Preoperatively, ultrasound and CT exams were relevant for tumor localization and the absence of metastasis; preoperative localization of the pancreatic exocrine tumor is a prerequisite for a successful resection of the tumor. The surgical procedure performed – open pylorus-preserving pancreaticoduodenectomy with standardized lymph nodes dissection, which has been proven to be equal to the classical pancreaticoduodenectomy in terms of tumor recurrence or long-term survival, and should therefore be considered the standard procedure for tumors of the pancreatic head, was associated with limited postoperative gastric hemorrhage and an delayed gastric emptying (ISGPS grade A), resolved by postoperative day 10; intraoperatively, tissue sampling was not limited to areas of macroscopically unequivocal tumor growth only, although is common knowledge that the prognostic significance of margins involvement in pancreatic cancer is currently unknown. By postoperative day 18, the patient was released.

Optic microscopic examination through pancreatic tumor lesions showed different grades of morphologic changes in the pancreatic histoarchitecture (Figure 2).
Different overviews through the pancreatic cancer tumor shows cells grown in a haphazard pattern associated with desmoplastic stroma, including polymorphonuclear inflammatory cells (Figure 3). Moreover, some edematous areas associated to the tumor lesions can be seen (Figures 4 and 6). Inside of pancreatic tumor lesions, very different ultrastructurally phenotypes of epithelial cells and stromal and inflammatory cells can be seen. Three main epithelial cell phenotypes can be distinguished (Figures 4, 6–8, 13 and 16). (1) The majority of epithelial cells have a large euchromatic and nucleolated nucleus embedded in a cytoplasm filled with many mitochondria, some endoplasmic reticulum and some cytoskeleton filaments. (2) A similar cell phenotype, but with hyaline cytoplasm, except for some short profiles of endoplasmic reticulum and scanty mitochondria can be seen. Intermingled with the above described cell phenotypes, another (3) epithelial cell phenotype exhibiting well-developed rough endoplasmic reticulum with various distribution of zymogen granules can be identified. Mention must be made that inside of some epithelial cells an amorphous-hyaline material is deposited (Figure 4, detailed in Figures 13 and 16).

Inside of the pancreatic ductular lesion, rare lumens with various diameters are formed (Figures 4 and 13). Small microvilli are oriented towards the lumen. Tight junction and desmosomal junction connect apical poles of adjacent epithelial ductular cells (Figures 5 and 8, inset). Tumor cells exhibit euchromatic nuclei, some of them being nucleolated. Large edematous areas and poly-

In the tumor area formed by atypical duct cells, independent shedding membrane vesicles can be identified (Figure 21 and inset). Sometimes, shedding membrane vesicles in the way to be delivered by a tumor cell can be detected. Moreover, tumor cell extensions are filled with lysosomes (Figure 22), meaning proteolytic enzymes. No basement membrane can be detected around the tumor cell extensions (invadopodia) and shedding vesicles.

Inside of tumor lesions, some small abnormal blood vessels appear collapsed (without lumen), but devoid of pericytes along some endothelial wall sectors (Figure 23).

Tumor exocrine pancreas lesions occupy extremely excessive part of the pancreas, detrimental to endocrine tissue counterpart. Our electron microscopic exploration of tumor pancreas allowed detection of at least three cell types (alpha, beta and delta cells) from the five major endocrine cell types, which are usually housed in normal endocrine pancreas. Indeed, very seldom endocrine islets can be encountered. When detected, at least three endocrine cell types (α, β, δ) can be identified (Figure 24). Because of the very high fragility of plasma membranes, dissipations of α- and β-endocrine cell plasma membranes may appear and, an illusive delimitation separates specific endocrine nucleoid-containing α-granules cell and a β-cell, as is depicted in Figure 25. The electron microscopic examination of β-cell tumor showed ultrastructurally granules varied considerably. Beta granules composed of one or several angular-shaped cores, suggesting a crystallloid component and a thin membrane surrounding them can be also detected (Figure 25).
Relevant infrastructural alterations in invasive pancreatic ductal adenocarcinoma

Figure 3 – Different overviews (A–D) through pancreatic cancer tumor shows cells grown in a haphazard pattern associated with desmoplastic stroma, including polymorphonuclear inflammatory cells (PMN). Large stars mark edematous areas. PDC: Pancreatic ductal cells; PAC: Pancreatic acinar cells; α: Pancreatic endocrine alpha cells; β: Pancreatic endocrine beta cells.

Figure 4 – An overview shows three high-grade neoplastic lesions areas (I, II and III). Interrupted lines follow a fibro-cellular tissue which seems to separate the three putative ductular dysplastic lesions with invasive behavior. Different epithelial cell phenotypes grown in a haphazard pattern can be identified. Amorphous-hyaline material can be seen inside of some epithelial cells (encircled areas). Elliptic areas mark zymogen-like granules. A large lumen (Lu) of one ductular zone eccentric located is visible. Edematous areas: Stars.

Figure 5 – Small microvilli (red arrows) are oriented towards the lumen (Lu) inside of a pancreatic ductular lesion. Yellow arrow marks the tight junction followed by a desmosomal junction (encircled area) between two-epithelial ductular cells.
Figure 6 – Neoplastic ductular areas. Tumor cells exhibit euchromatic nuclei, some of them being nucleolated. Large edematous areas as well as polymorphonuclear cells (PMN) can be identified.

Figure 7 – Putative pancreatic ductal cells (PDC) intermingled with acinar cells (AC) and endocrine alpha (α) and beta (β) cells. mv: Microvilli; z: Zymogen granules; z-l: Zymogen-like granules; M: Mitochondria; RER: Rough endoplasmic reticulum; csk: Cytoskeleton. In inset: Overview for the Figure 7. AC: Acinar cell; White arrows: Zymogen granules; Red head arrow: Microvilli; α: Endocrine alpha cells.
Figure 8 – Inside of putative duct cells (PDC) small primary lysosomes of different sizes zymogen-like (encircled areas) coexist with many mitochondria. One of putative duct cell exhibits an autophagic area (green star). Small cell extensions are projected inside of lumen (Lu), detailed in inset (red arrows). In inset, close to the apical pole, a zymogen-like granule can be seen (encircled area).

Figure 9 – Excessive dilated rough endoplasmic reticulum and mitochondria with destroyed cristae (green stars) can be seen.

Figure 10 – Excessive vacuolized/edematous cytoplasm areas push eccentrically the nucleus (N) and the cytoskeleton (Csk).

Figure 11 – Plasma membranes of adjacent tumor cells (affronted red arrows) appear zonally illusive (black arrows), herniate deeply inside of cytoplasm (encircled area) or perform plasma membrane recombination (elliptic area).

Figure 12 – Severe alterations of tumor cell plasma membranes. Extensive sectors of plasma membranes belonging to few adjacent tumor cells appear illusive (red arrows). Small stars mark the leakage materials of desmosomal junctions. An autophagy area is marked by a large star. Csk: Cytoskeleton.
Figure 13 – Overview on the presumptive acinar area intermingled with putative duct area. Most nuclei are euchromatic with large nucleoli (nu). Both presumptive acinar cells and putative duct cells exhibit primary and atypical small zymogen granules (encircled areas) and extensive areas of autophagy (stars). Two acinar lumens (Lu) with some microvilli are visible (white square – detailed in Figure 14). f: Filaments inside of a lumen can be detected.

Figure 14 – Apical poles of few acinar cells exhibit microvilli (red arrows) oriented towards the lumen. Microvilli have a microfilamentous core and are surrounded by glycocalyx (yellow arrows). A tight junction (black arrow) and a desmosomal junction (white square) can be detected. Inset A: Few acinar microvilli (red arrows). Some cross-sectioned microvilli (black squares) are surrounded by glycocalyx (yellow arrows). Inset B: A desmosome and associated cytoskeleton (csk) can be seen. Head arrows mark desmosomal midline.

Figure 15 – Normal pancreatic acinus represented by a centroacinar cell with euchromatic and nucleolated nucleus together with few apparent normal acinar cells with numerous zymogen granules (z) distributed at the apical pole, close to the lumen (Lu). Acinar cells are rich in rough endoplasmic reticulum (RER) and exhibited microvilli (mv). M: Mitochondria.
Figure 16 – A centroacinar cell with electron-lucent cytoplasm (poor in organelles) is surrounded by putative acinar cells severely affected by extensive autophagic areas (stars). Very few and small zymogen granules (encircled areas) can be seen inside of acinar cells. Acinar lumen is absent.

Figure 17 – A cytoplasmic sector of an acinar cell with numerous mitochondria with altered cristae (white arrows) sequestered by dilated areas of rough endoplasmic reticulum (blue arrows). A hyaline-amorphous area can be detected inside of an altered mitochondria (yellow star). z: Zymogen granules. In inset: Detail for Figure 16. Altered mitochondria (aM).

Figure 18 – A net of rough endoplasmic reticulum (blue arrows) sequestered apparently normal two mitochondria (M) and a large altered mitochondria (green star). The large mitochondria exhibit an external membrane (red arrow) but disorganized cristae. A filamentous material (white arrow) eccentric located can be seen inside of altered mitochondria. z: Zymogen.

Figure 19 – A huge fibrillar-hyaline-amorphous deposit material (star) inside of an epithelial cell from the tumor area is attached to the nuclear content.

Figure 20 – A very large fibrillar-hyaline-amorphous deposit material is firmly attached to the nuclear envelope. Inset A: Detail from the fibrillar material with a net appearance. Inset B: Detail from the fibrillar material attached to an hyaline part (star) of the deposit material.
Figure 21 – Inside of a tumor area formed by atypical ductal cells, independent shedding membrane vesicles (stars) are visible. Shedding membrane vesicles in the way to be delivered by a tumor cell can be detected (elliptic area, detailed in inset).

Figure 22 – Individual shedding membrane vesicles (red stars) adjacent to a tumor cell extension (black star) filled with lysosomes (encircled areas) can be seen.
Relevant infrastructural alterations in invasive pancreatic ductal adenocarcinoma

Figure 23 – Collapsed small blood vessels represented by endothelial cells (EC) surrounded by basement membranes (red arrows) exhibit interendothelial junctions (black arrow) but no lumen. A pericyte (PC) with characteristic subplasmalemmal small densities (head arrows) with a proper basement membrane (blue arrow) accompany the abnormal blood vessels. Red stars mark redundant basement membranes.

Figure 24 – An overview through an endocrine area: α, β and δ cells can be identified.

Figure 25 – Illusive delimitation (white interrupted line) separates specific endocrine nucleoid-containing α-granules cell and a β-cell showing a crystalline β-granule (yellow arrow).

Discussion

A tumor is an organ [23], a complex multicellular ecosystem represented by (1) neoplastic genetic altered cells and (2) tumor stroma represented by (a) different cell types (fibroblasts, fibrocytes, mast cells, inflammatory cells, endothelial cells, pericytes, naked or myelinated nerves, etc.) as well as (b) extracellular matrix (basal lamina, collagen and elastic fibers and soluble molecules). Autocrine and paracrine factors offer the necessary support for tumor growth, a permanent cross talk between malignant cells and peritumoral stroma being necessary [24].

TEM analysis demonstrates that pancreatic cancer tumor cells grown in a haphazard pattern associated with polymorphonuclear inflammatory cells. Mention must be made that quite often some edematous areas associated to the tumor lesions can be detected. Concerning the most striking altered aspect of pancreatic histoarchitecture is the presence of putative ductal cells intermingled with presumptive acinar areas inside of a tumor lesion (Figures 3, 4 and 7), but neoplastic ductal cells with invasive growth are by far prevalently (Figure 6). Indeed, tumor cells with an ultrastructural aspect remembering the ductal phenotype (many mitochondria and a rich cytoskeleton can be seen inside of cytoplasm) are intermingled with cells whose ultrastructural aspect suggests acinar phenotype (cytoplasm is mostly occupied by rough endoplasmic reticulum and zymogen granules) (Figures 4, 7, 8, and 13).

The normal pancreatic ductal system starts with centroacinar cells, small cells embedded within acini, and proceeds through progressively larger ducts, termed intercalated (the smallest unit of the ductal system, starting in the acinus), intralobular, interlobular, and major ducts in increasing order of size. The ductal system serves as a conduit for pancreatic exocrine secretions; in addition, it also modifies the pH of pancreatic juice by secreting sodium bicarbonate, water (buffering of pancreatic juice stabilizes the exocrine proenzymes). The centroacinar cell constitutes the beginning of the intercalated duct draining the acinus and is located within the acinus, near its center. Due to the absence of zymogen granules and the paucity of the organelles, cytoplasm of centroacinar cells appears pale comparing with acinar cells (Figures 15 and 16). The cells of intercalated ducts are flattened, cuboidal, similar to centroacinar cells with a small number of microvilli on the luminal surface. Solitary modified cilia (kinocilia) project from the luminal surface [15].

The origin of pancreatic cancer is still a matter of debate [12]. So far, as for many other solid tumors, in the case of pancreatic cancer yet is not clear if the tumor cell originate from resident stem cells. Recently studies suggest that cells with stem activity may arise by transdifferentiation or dedifferentiation of other cell types. This means that some cells have the potential to be stimulated and differentiate to assume a stem cell role (so-called facultative stem cells) [1, 25].

Pancreatic ductal/duct cells have the capacity to differentiate into both exocrine and endocrine lineages [1, 25]. Acinar, ductal or islet cells have been hypothesized as the cell of origin [1]. It seems that there is no unique cell of origin for PDAC.

The parenchyma of the pancreas develops from the
dorsal and ventral primordia within the duodenal endoderm at four weeks of gestation [26–28] that later fuse to become a single organ [1]. During the embryonic development of the pancreas, all exocrine and endocrine cells are derived from the common precursor cells, the stem cells, which are retained in the adult pancreas and are the foundation for cell renewal and tumors [29]. Stem cells require some mutations to undergo malignant transformation. One the other hand, the replacement of one mature cell type with another has been identified in a large variety of cancers [30]. Intermediate cells (hybrid one mature cell type with another has been identified in transformation. One the other hand, the replacement of are the foundation for cell renewal and tumors [29]. Moreover, cilia were absent from mouse PanIN cells in human pancreatic ductal adenocarcinoma (PDAC).

Moreover, cilia were absent from mouse PanIN cells in human pancreatic ductal adenocarcinoma (PDAC). Our TEM investigations demonstrate that inside of the tumor cells does not exhibited neither a pure ductular or ductal nor a pure acinar phenotype, but tumor lesions represented by neoplastic ductal cells with invasive growth are by far prevalent. Our electron microscopic observations also underline the high plasticity of the pancreatic parenchyma cells.

Acarinar cell carcinoma represents 1–2% from all exocrine pancreatic (non-endocrine) tumors [34–36]. There are acinar cell carcinoma variants: mixed acinar-ductal carcinoma, mixed acinar-endocrine carcinoma or mixed acinar-endocrine-ductal carcinoma [36].

Interestingly, our electron microscopic examination suggests that neoplastic lesions are represented by putative ductal cells intermingled with presumptive acinar areas. To date, there is a matter of discussion concerning acinar-ductal metaplasia (ADM) as important component of neoplastic transformation. At least in the mouse, ADM is an important precursor to PanIN lesions, and PAN lesions progress to PDAC [28].

Our TEM investigations demonstrate that inside of tumor pancreas lesions both centroacinar and ductal cells do not express basal body cilium and no cilium projects towards the lumens formed by apical pole of either presumptive acinar or putative ductal areas from tumor pancreas lesions. Instead, some short microvilli can be identified, as is depicted in Figures 5, 8 (inset) and 14. Seeley et al. [37] also reported that primary cilia were absent in both pancreatic cancer cells and PanIN lesions in human pancreatic duct adenocarcinoma (PDAC). Moreover, cilia were absent from mouse PanIN cells in different models of PDAC driven by an endogenous oncogenic K-RAS allele. Interestingly, inhibition of K-RAS effector pathways restored ciliogenesis, raising the possibility that ciliogenesis may be actively repressed by oncogenic K-RAS.

In normal human mouse and rat pancreas, centroacinar and ductal cells as well as some islet cells may exhibit primary cilia [15, 29, 37–40]. On the other hand, no primary cilia was ever observed in the acinar cells of mouse and rat pancreas [40]. Primary cilia are non-motile sensory organelles involved in signal transduction [41, 42]. Recently, Wilsch-Bräuning et al. [43] demonstrates that re-establishing the baso-lateral primary cilium preceding neural progenitor delamination – a hallmark of cerebral cortex development. Ablation of primary cilia might cause up-regulation of the Hedgehog (Hh). Hh signaling is involved in regulation of pancreatic epithelial plasticity by reprogramming mature exocrine cells (including pancreatic tumor formation) [44]. Ciliary abnormalities are implicated in different epithelial morpho-functional disturbances known as ciliopathies [45, 46]. Cano et al. [47] reported that absence of cilia in pancreatic cells results in chronic pancreatitis and cystic fibrosis. Ciliol ciliation may prevent centroacinar duplication and the formation of mitotic spindle. Primary cilia are normally absent in rapidly proliferating cells due to their resorption during cell cycle progression; proliferating cells are unable to ciliate [37, 48].

It was considered that acinar cell carcinomas derives from transformed acinar cells, but in some cases, a certain cell population acquire the ability to differentiate into endocrine cells, whereas the other cell maintain their acinar phenotype so that mixed acinar-endocrine cell tumors can be developed. Moreover, incomplete differentiation of some acinar cell carcinomas towards ductal adenocarcinoma can be recorded [34]. Our electron microscopic observations showed that apical poles of presumptive acinar cells exhibit microvilli towards the lumen and tight junction connects adjacent acinar cells, as it is described also in acinar cell carcinoma [36]. Nonetheless, mention must be made, when compared typical ultrastructural aspect of the human pancreatic cell carcinoma [34] or canine acinar cell carcinomas [49], in our investigated case most acinar cells are abnormal: rough endoplasmic reticulum is less polarized (it occupy apical pole, as is depicted in Figures 7 and 13), zymogen granules are scanty, many being smaller than normal (atyypical zymogen-like granules in Figures 13 and 16) and huge areas of autophagic areas occupy the most part of cytoplasm (Figure 13). In some tumor area, ultrastructural aspect suggests that cells depicted in Figure 8 can be assimilated to the presumptive ductal cell carcinoma phenotype: inside of cytoplasm, many mitochondria co-exist with zymogen-like granules. Satake et al. [50] also reported that occasionally, even zymogen-like granules were absent suggesting a dedifferentiation of acinar cells toward the ductular phenotype.

In our investigated case, many autophagic figures can be seen, especially affecting cells from presumptive dysplastic pancreatic acini (Figures 13 and 16).

Extensive areas of cytoplasm are filled with secondary lysosomes, including residual bodies, and large lipofuscin
granules [not shown], so that, at the end, huge deposits of hyaline fibrillar material can be seen inside of affected cells (Figures 19 and 20). Concerning the biogenesis of such deposit material, our electron microscopic investigation demonstrates that often mitochondria are wrapped by dilated cristae of rough endoplasmic reticulum. Sometimes, as is depicted in Figure 17, together with mitochondria, a lysosome/zymogen granule is wrapped by endoplasmic reticulum endomembrane (mitophagy). Trapped mitochondria exhibit altered disorganized cristae (Figures 17, 17–inset, and 18). It seems that at the end, the affected mitochondria follow a complex transformation so that a kind of hyaline-fibrillar material complexed with lipofuscin is constituted (Figures 19 and 20). Fibrillar material may form a kind of net (Figures 20 and 20–insets A and B).

Electron microscopic examination revealed that inside of pancreatic tumor cells [ductal adenocarcinoma cells] many mitochondria are entrapped and sequestered by endoplasmic reticulum. Some mitochondria appear to preserve normal ultrastructure but other mitochondria exhibit different degree of ultrastructural alterations. The disarrangement and distortion of cristae and accumulation of a hyaline-amorphous material in a dense matrix are very frequently encountered aspects in tumor pancreas (Figures 17 and 18). Novotný et al. [51] consider that functionally such mitochondrial changes in adenocarcinoma of the pancreas presume the presence of hypoxia-tolerant and hypoxia-sensitive cancer cells.

Many tumor cells exhibit a well-developed rough reticulum with and, sometimes, only scanty zymogen granules. Microvilli and cellular projections towards the lumen were scant. Mention must be made about the existence of some cells with a strong rough endoplasmic reticulum but missing zymogen granules.

Autophagy is known to promote survival in response to nutrient starvation/deprivation, but can also promote cell death, depending on the tissue type and circumstances [5, 21, 52]. Autophagy is a dynamic process, during an organelle, for example mitochondria is surrounded by the endoplasmic reticulum as is depicted in Figures 17 and 18. The membrane surrounding the organelle then fuses with a lysosome to produce autophago-lysosome. Deciphering the meaning of the extensive process of autophagy inside of pancreatic tumor examined in this study, we may underline that in literature there are conflicting opinions. The role of autophagy in cancer is very complex. Autophagy may play an important role in carcinogenesis as well as in eliminating cancer cells [5, 21]. Human and mouse models shows that inactivation of autophagy can promote tumorigenesis [53]. Autophagy has been shown to be regulated by reactive oxygen species (ROS). Conversely, loss of autophagy can induce increased ROS leading to DNA damage. Taking in consideration that inflammation and ROS are associated with both RAS transformation and the initiation of PDAC, Yang et al. [5] consider that is possible the elevated basal autophagy in PDAC may also serve as an adaptation to prevent the accumulation of genotoxic level of ROS and, consequently, restrain DNA damage in PDAC cells thereby allowing sustained tumor growth.

In our pancreatic cancer case, TEM investigation clearly shows that a plethora of the epithelial cells from the tumor lesions contain large areas of autophagy leading to the pleomorphic inclusions (composite bodies). Fibrillar/ filamentous inclusions (FIs) are frequently associated with hyaline-amorphous material, lipid droplets and secondary lysosomes (Figures 18–20).

Fibrillar/filamentous inclusions (FIs) are unusual shaped cytoplasmic inclusions, found mostly in acinar cell carcinomas of the pancreas, considered an abnormal zymogen granule-type, a recapitulation of the fetal zymogen granules [36]. Pasquinelli et al. [54] described such inclusions in acinar, centroacinar, and small duct epithelial cells from non-neoplastic pancreas, found as well in tumor cells from a mixed acinar-endocrine pancreatic carcinoma. Immunogold labelling indicates that FIs are aggregate of intermediate filaments immuno-reacting with some anti-cytokeratins and with anti-vimentin monoclonal antibodies.

High plasticity/fragility of cytomembranes, including plasma membrane is a hallmark for the malignant tumor cells [55-57]. Plasma membrane is a dynamic mosaic of distinct micro-domains often corresponding to specific infrastructures and unique molecular composition, properties and functions. On may speculate that long exposure of tumor cells to a lot of paracrine factors (cytokines) and enzymes produced by extravasated inflammatory cells (Figures 3 and 6) leads to the disorganization of surface domains or even focally plasma membrane dissolution/destruction, already fragile by itself because of being malignant tumor cells (Figures 11 and 12). This may lead to the pseudo-synctia formation.

Like many other type of genetic altered malignant cells [58–61], pancreatic tumor cell shed membrane vesicles inside of the peritumoral stroma as is visible in Figures 21 and 22. Tumor cell shedding membrane extracellular vesicles transfer inside of ECM membrane attached receptors, growth factors, nucleic acids (DNA, mRNA, microRNA), proteins, lipids, almost all molecules involved in cell signaling [62] or are causative of cancer progression [63–65]. Uptake of tumor cell derived vesicles (oncosomes) by initially non-malignant (genetic non-altered) neighboring cells may change cell phenotype and become a malignant tumor cell (genetic altered phenotype) [61, 64, 66]. Moreover, according with other opinions [67], we consider that shedding membrane vesicles offer false targets for immune cells, a modality to escape of immune surveillance. Other microvesicles contain lysosomes (Figure 22), meaning proteolytic enzymes whose deliverance contribute to extracellular matrix, including basement membrane destruction. Moreover, down regulation of cell–cell and cell–extracellular matrix adhesions might result in loss of cell polarization and aberrant cell behavior, including the promotion of cell migration and consequently invasive growth, a pre-requisite for ectopic places secondary tumor formation (metastasis).

Endocrine component of the pancreas represents approximately 1% of the whole pancreatic mass [68], but it plays a crucial role in regulating metabolic function, mainly the glucose metabolism. The concentration of glucose in blood stream is a very important parameter of human body homeostasis.

In our investigated case, tumor lesions of exocrine
pancreas occupy extremely excessive parts of the pancreas, detrimental to endocrine tissue counterparts so that very seldom-endocrine islets can be encountered. When detected, at least three endocrine cell types (α, β, δ) can be identified (Figure 24). Because of the very high fragility of plasma membranes, dissolutions of the α and β endocrine cell plasma membranes may appear and, an illusive delimitation separates specific endocrine nucleoid-containing α-granules cell and a β cell, as is depicted in Figure 25.

Traditional histological descriptions of the pancreas, dividing pancreas into two separate entities as exocrine and endocrine pancreas, seems to be incorrect. Along the time, new observations concerning existence of some relationships between islet and acinar tissues lead to the hypothesis of so-called “islet acinar axis”. New data concerning relationships, including existence of topographical associations between Langerhans islets and ducts were accumulated. All these above observations stated that pancreas is an integrated, well-tuned organ, so that an “endocrine-exocrine-ductal axis” concept can be considered [69]. In this context, we have to emphasize that in our case, scattered endocrine cells can be detected within the tumor lesions represented by presumptive exocrine parenchyma and the putative duct epithelium (Figure 7, including inset as overview). Isolated pancreatic endocrine cells all four major type secreting glucagon (α cells), insulin (β cells), somatostatin (δ cells) and pancreatic polypeptide (PP cells) within the exocrine parenchyma and the duct epithelium have been reported [69]. Endocrine cells associated to the ductal adenocarcinoma of the pancreas were also reported [34].

Treatment options for pancreatic cancer are very limited. When possible, surgical removal of the tumor is a common treatment. Otherwise, only palliative actions can be applied [9].

Conclusions

Our electron microscopic observations underline the high plasticity of the pancreatic parenchyma cells. The tumor pancreatic cells does not exhibit neither a pure ductular or ductal nor a pure acinar phenotype, but tumor lesions represented by neoplastic ductal cells with invasive growth are by far prevalently. The high fragility (extensive dissolutions) of plasma membrane of tumor cells may results in pseudo-synctia formation. Plasma membranes shed membrane vesicles. Extravasated inflammatory cells contribute to the dramatic and extensive destructive areas of epithelial cells as well as tumor-stroma counterpart. Electron microscopy investigations are essential to diagnose pancreatic cancer particularities/subsets.

Conflict of interests

The authors declare that they have no conflict of interests.

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