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Microcalcifications in stone-obstructed human submandibular gland are associated with apoptosis and cell proliferation

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ABSTRACT

Objective: Human submandibular gland (SMG) stones are associated with inflammation, fibrosis and microcalcifications in the surrounding tissues. However, there is little information about the accompanying cell injury-repair process, apoptosis, and cell proliferation. The purpose of this study was to investigate such an association and its clinical significance.

Design of study: Mid-gland paraffin sections of human SMGs ("stone glands") and normal SMGs ("non-stone glands") were subjected to stains for general histology (hematoxylin and eosin), fibrosis (Masson's trichrome), and calcification (alizarin red) and to immunohistochemistry for proliferative activity (Ki-67), and apoptosis (Caspase-3). Tissues were assessed for areas of inflammation, calcium deposition, and fibrosis, and for cycling and apoptotic cells.

Results: Acini were atrophic and proportionately fewer in lobules with fibrosis in stone glands. Additionally, stone glands had intraluminal calcifications (microliths) in scattered excretory and striated ducts and blood vessel walls. Areas of inflammation and fibrosis were small and uncommon, and calcifications were not seen in non-stone glands. Proliferating and apoptotic cells were common in the main duct of stone glands where ciliated and mucous cell hyperplasia and stratified squamous metaplasia had occurred, uncommon in the main duct of non-stone glands, and uncommon in all other parenchymal elements of both stone and non-stone glands.

Conclusion: Stone obstruction in the main excretory ducts of SMG resulted in progressive depletion of acini from proximal to distal lobules via calcification, inflammation, fibrosis, and parenchymal cell atrophy, apoptosis and proliferation. Interlobular duct microliths contributed to this depletion by further provoking intralobular inflammation, fibrosis, and acinar atrophy.

1. Introduction

Calcium phosphate (CaP) crystals incite the process of calcium stone formation as well as ectopic calcification. Such calcification displays some similarities with the mechanisms of bone formation, where bone related factors have been shown to play an important role in vascular and renal calcification (Jia, Wang, & Tang, 2014; Schweighofer, Aigelsreiter, & Trummer, 2016). Moreover, calcification can be the body's protective response to injury, as well as part of a natural inflammatory reaction to infection, trauma, or autoimmune disorders. Tumor tissues (either cancerous or noncancerous) also have been shown to contain areas of calcification (Khan et al., 2010), however the link between those phenomena to support a mechanism is unknown. Similarly, the inflammatory response can be triggered by ectopic calcifications (Fukuyo, Yamaoka, & Sonomoto, 2014), which can then release several inflammatory cytokines such as IL-6 and TNF-alpha to induce the signaling pathways leading to osteoblast differentiation, essential for calcium biomineralization (Fukuyo et al., 2014; Watson et al., 1994). Additionally, calcium crystals are able to generate a proinflammatory response (Harrison, Triantafyllou, Baldwin, & Schäfer,

Abbreviations: CaP, calcium phosphate; SMG, submandibular gland; FFPE, formalin fixed paraffin embedded; H & E, hematoxylin and eosin; DAB, 3,3 diaminobenzidine; CA, carbonic anhydrase; CMBA, 9,10-dimethyl-1,2-benzanthracene

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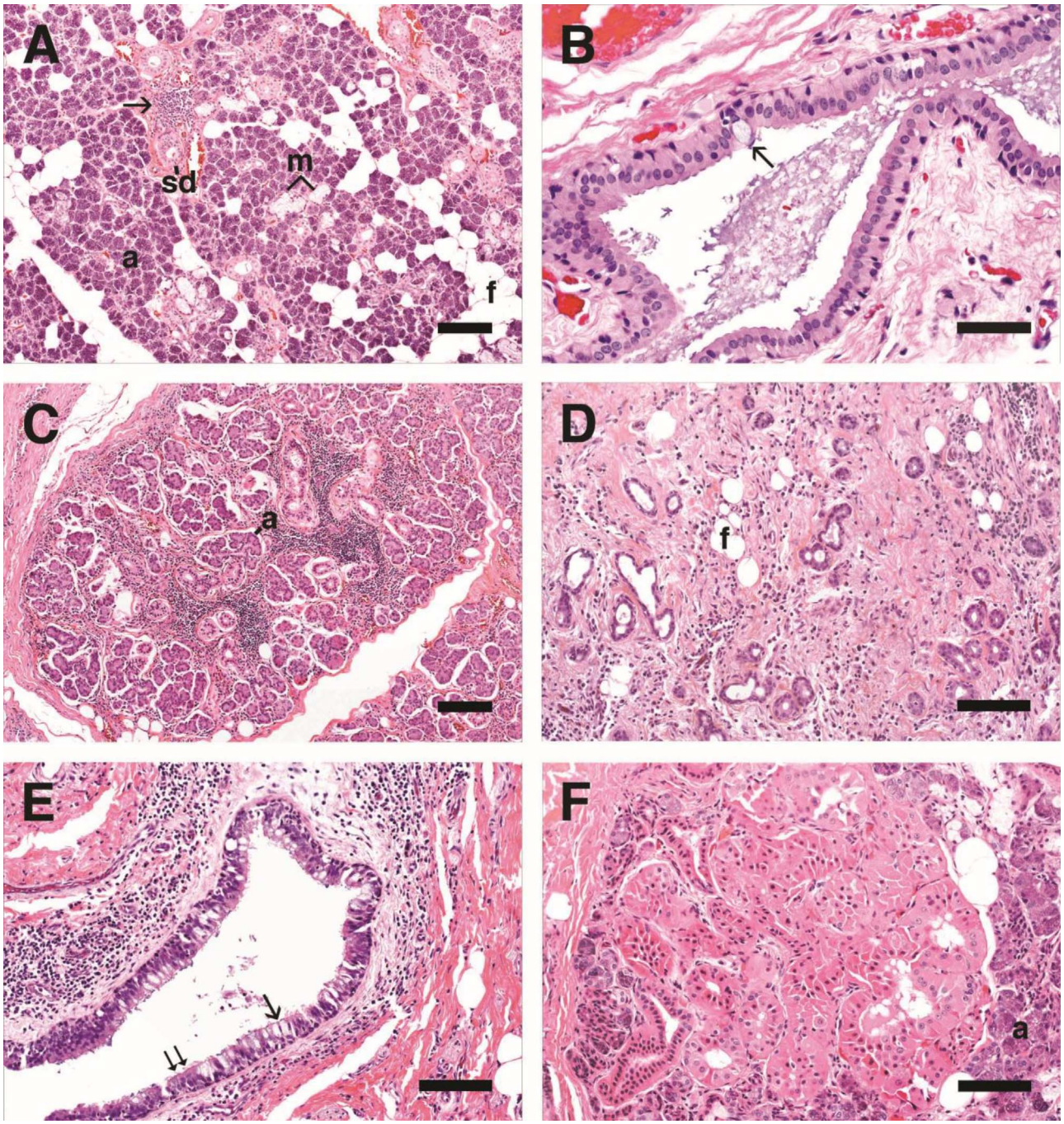

Fig. 1. General histologic features. A, B, Normal glands; C-F, Stone glands. A. Serous acini are almost filled with secretory granules (purple dots). There is a focus of periductal infiltration of lymphocytes (arrow). B. This segment of main excretory duct has a solitary mucous ("goblet") cell (arrow). C. Lobule with atrophic (few, if any, secretory granules) acini and a moderate to heavy infiltration of inflammatory cells, mostly lymphocytes and plasma cells. D. Advanced stage lobular atrophy, in which acini appear to be absent and ducts and duct-like structures are sparsely distributed in a fibrotic (pink collagen fibers) stroma. E. The epithelium of this large excretory duct shows extensive hyperplasia of goblet cells (arrow) and ciliated columnar (double arrow) cells. There is a moderate collection of lymphocytes and plasma cells in the surrounding stroma. F. Oxyphilic (oncocytic) metaplasia. Some of the oxyphilic cells (deep pink cytoplasm), in this nodule retain the general structure of striated ducts. a = serous acini; f = fat (adipose) cells; m = mucous acini; sd = striated ducts. Hematoxylin and eosin stains. Magnification bars: A, C = 150 μm; D-F = 100 μm; B = 50 μm.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1993; Watson et al., 1994) in macrophages, which ingest CaP crystals, secrete the cytokines TNF-alpha, IL-1-beta, and IL-8 (Nadra, Mason, & Philippidis, 2005). Since CaP crystals are able to trigger the secretion of inflammatory cytokines which themselves are able to trigger the formation of calcifications, it is likely that there is a positive feedback loop between calcification and inflammation (New & Aikawa, 2011).

Likewise, links between calcification and other physiological conditions such as fibrosis have also been described, particularly in renal tissue (Evan, Lingeman, & Coe, 2005), where CaP stones have been linked to loss of nephrons (Coe, Worcester, & Evan, 2016). Moreover, calcium crystals are also associated with fibrosis, calcification and cell proliferation (Hayes, Brodie, O'Doherty, & Quinn, 2013; Morgan, Cook, & McCarthy, 2005; Weon, Park, & Kim, 2012). Furthermore, increased cell turnover (i.e., increased proliferation and apoptosis) can be a contributing factor in tumorigenesis (Liu, Edgerton, & Thor, 2001). However, such cell injury-repair processes accompanied by increased cell proliferation and apoptosis have not been connected to stone-