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Hypothesis

Unmasking the gray zone of hyalinization with a proposed classification of oral hyalinizing lesions

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ABSTRACT

Histopathology encompasses a variety of formations projected as different shades of pink and purple. The appearance of every specific substance is attributed to the pathogenesis or process of its very being. Hyalinization is one such routinely encountered appearance, the pathogenesis of which remains untouched. Thus, an attempt has been made to unveil the gray zone of hyalinization as well as clinically correlate it to oral lesions. A classification for oral lesions showing hyalinization has also been proposed.

KEY WORDS: Histopathology, hyalinization, pathogenesis, oral lesions

INTRODUCTION

The word "hyaline" is derived from the Greek word *hyalos*, denoting a colorless, glassy, transparent substance [1]. It is a descriptive histological term for homogeneous, structureless, eosinophilic material in hematoxylin and eosin stained tissue sections and does not refer to any specific substance. It simulates an acellular and avascular amorphous mass which necessitates its differentiation from its mimickers such as amyloid and fibrin under light microscopy. Hyalinization is often preceded by an alteration within the cells or extracellular region. The different causes of hyalinization can be delineated as fibrosis, secretory products of cells, degenerative changes, and deposition of basement membrane (BM) like material and blood derived fibrin. Thus, the hyaline deposits or the non-amyloid eosinophilic substances are not identical and vary to a large extent in their chemical composition.

The amorphous eosinophilic material, a characteristic light microscopic feature of various tumors is predominantly the BM like material, i.e., collagen IV and laminin secreted by neoplastic cells. However, the modus operandi behind hyalinization has not yet been analyzed. It has been proposed that the process of hyalinization begins intracellularly with the deposition of hyaluronan, and its concentration defines the pattern of hyalinization as reported in few malignant neoplasms [2,3].

The slacking of collagen strands, ill-defined periodicity, and disappearance of cross banding are the features associated with deteriorated collagen fibrils in hyalinization. An abnormal crosslinking in collagen fibers results in the formation of hypocellular areas made up of homogenized collagen fibers. Hyalinized collagen has been found to be modified biochemically. Denduchis *et al.* reported a progressive increase in the hydroxyproline concentration with increasing severity of hyalinization [4].

This discussion is limited to hyalinization associated with fibrosis.

AN INSIGHT INTO HYALINIZATION

Thorough search of the pertinent literature did not reveal any available study focusing on the pathogenesis of hyalinization. In the author's opinion and based on light microscopy, histochemistry and immunohistochemical reactivity of hyalinized areas, as reviewed from the literature, hypertrophic scar/keloid may perhaps prove a suitable prototype to underpin the molecular aspects of hyalinization. The following section presents a hypothesis in relation to the previously acknowledged factors in different disease processes.

Among the three types of transforming growth factor (TGF), TGF-β1, and TGF-β2 are expressed in adults while expression of TGF-β3 is seen during embryological development [5]. TGF-β1/2 plays an indispensable role in downregulating the collagenolytic activity and promotes collagen deposition. It stimulates the transcription of collagen I and impedes the transcription of collagen-degrading enzymes [6]. The surplus collagen in keloids is the consequence of increased expression of TGF-β1 and diminished degradation of collagen along with downregulation of the inhibitory effect of SMAD6 and SMAD7 proteins [Figure 1] [7].

Other than hypertrophic scars and keloids, hyalinization has been reported in various other benign and malignant neoplasms of different cellular origins. An aberrant expression of TGF-β has been found in the tumoral tissue [8-14]. For instance, stromal hyalinization is one of the characteristic microscopic features of ovarian clear cell carcinoma (CCC) [3]. Genetic analysis has shown that TGF is frequently mutated in ovarian carcinomas [15,16]. This implies that mutations associated with this growth factor could be the underlying cause of excess collagen deposition leading to hyalinization. On the other end of the spectrum, hyalinization is also featured in oral submucous fibrosis (OSMF), a potentially malignant disorder affecting individuals habituated to areca nut chewing; and systemic sclerosis characterized histologically by hyalinized collagen in lamina propria [17]. Molecular studies have identified TGF-β as the prominent mediator in the pathogenesis of OSMF [18]. The expression of TGF-β1 and TGF-β2 has been found to increase with the grades of OSMF [19,20]. Moreover, the characteristic juxta-epithelial hyalinization becomes distinct with increasing severity of OSMF. This highlights the possible link between the TGF-β expression and hyalinized areas in OSMF. The levels of cytokine and interferon-γ have been found to be downregulated [21] while expression of the profibrogenic growth factor, IGF-I is upregulated in keloids, OSMF, and systemic sclerosis [22,23]. Hence, the pathogenesis of hyalinization could be speculated as the interplay between different growth factors and cytokines.

Further research is needed to analyze the biochemical changes in hyalinization such as - Is there any abnormal proteoglycan or glycosaminoglycan (GAGs) in the hyalinized areas? Is hyalinized appearance secondary to increased total proteoglycans leading to water retention? and Does GAGs modulate the growth and differentiation of fibroblast synthesis leading to hyalinization in a complex interplay of tumor microenvironment?

STAINING CHARACTERISTICS

Histochemical Staining

Hyalinization appears homogeneously pink on routine staining. Its distinction from other similar appearing structures, for instance amyloid, is done histochemically. Special stains such as Periodic Acid-Schiff (PAS), Congo red, Thioflavin T, or

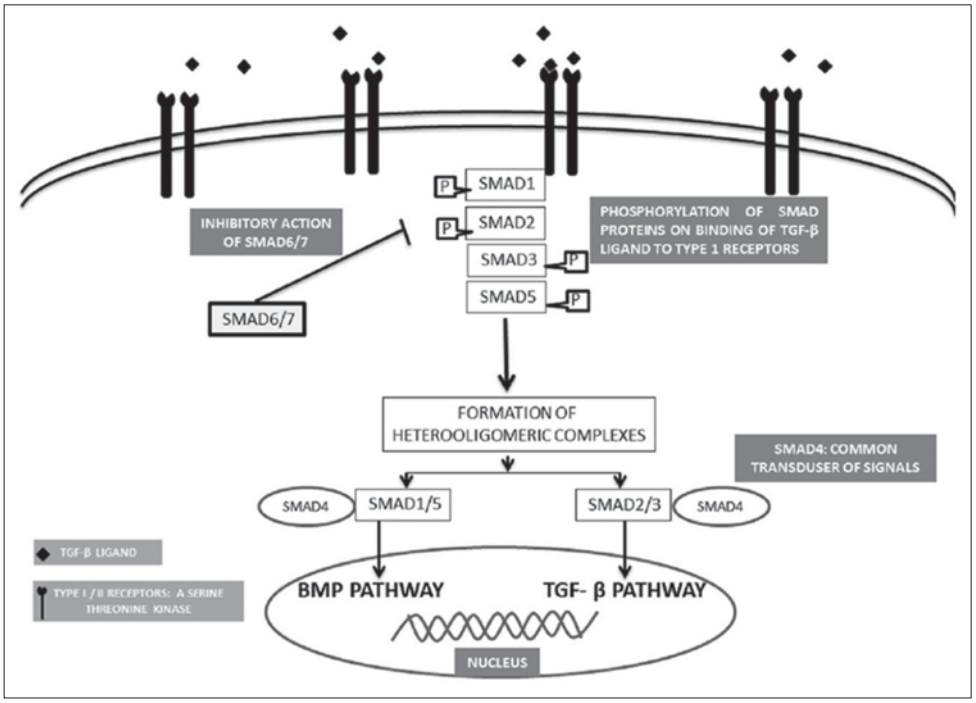

Figure 1: Crosstalk between transforming growth factor-β1 and "SMAD" protein in the pathogenesis of hyalinization

Thioflavin S are used to differentiate amyloid from hyalinized areas. PAS positivity is seen only in hyalinized areas. Amyloid stains salmon pink with Congo red under light microscopy while shows apple green birefringence under polarized light. In contrast, hyaline deposits show low affinity. Thioflavin T/S stain in the presence of amyloid show strong yellow, green, or blue fluorescence depending on the filter used while hyaline areas and hyalinized collagen are weakly reactive. However, in some cases, some overlap of the staining pattern might be expected [24].

Immunohistochemical Staining

Immunohistochemically areas of hyalinization show strong diffuse reactivity to collagen IV and laminin [3,4].

To conclude, the process of hyalinization in physiology as well as pathology can be recognized as cellular to extracellular matrix interactions. Knowledge of hyalinization with its pathogenesis is limited and the principal question still remains untouched. What is the chief triggering stimulus for the cascade of events responsible for hyalinization? Is there any threshold for the expression of fibrogenic growth factors and various cytokines? Is there any genetic predisposition secondary to HLA associations or mutations in different benign and malignant neoplasms?

PROPOSED CLASSIFICATION FOR ORAL LESIONS SHOWING HYALINIZATION [25-29]

Based on the Behavior and Growth of the Tumor

I. Benign:
   a. Ameloblastoma
   b. Ameloblastic fibroma
   c. Pleomorphic adenoma
   d. Schwannoma
   e. Nodular fasciitis
   f. Leiomyoma
   g. Lichen sclerosus
   h. Systemic sclerosis/scleroderma
   i. OSMF.

II. Malignant:
   a. Basaloid squamous cell carcinoma
   b. Mucoepidermoid carcinoma
   c. Adenoid cystic carcinoma
   d. Polymorphous low-grade adenocarcinoma
   e. Fibrosarcoma
   f. Hemangiopericytoma
   g. Leiomyosarcoma
   h. CCC-not otherwise specified (CCC-NOS)/hyalinizing CCC (HCCC).

Based on the Tissue of Origin

a. Epithelial origin:
   i. Odontogenic:
      • Ameloblastoma
      • Ameloblastic fibroma.
   ii. Non-odontogenic:
      • Basaloid squamous cell carcinoma
      • OSMF.
   iii. Cutaneous lesions:
      • Lichen sclerosus
      • Systemic sclerosis/scleroderma.
   iv. Salivary gland origin:
      • Pleomorphic adenoma
      • Polymorphous low-grade adenocarcinoma
      • Mucoepidermoid carcinoma
      • Adenoid cystic carcinoma
      • CCC-NOS/HCCC.

b. Connective tissue origin:
   i. Neural:
      • Schwannoma.
   ii. Smooth muscle origin:
      • Leiomyoma.
   iii. Fibrous tissue origin:
      • Nodular fasciitis
      • Fibrosarcoma.
   iv. Vascular origin:
      • Hemangiopericytoma.

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