Dysplastic Proliferation of Odontogenic Epithelium on the Xenograft Bones Inserted for Dental Implant

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A 57 years old female received xenogenic bone graft for the extraction socket augmentation of right maxillary molars and for the sinus floor elevation six months ago. The bone graft sites were healed uneventfully and showed marked radiopacity in the postoperative X-ray view. Before dental implant insertion the bone biopsy was made using trephine bur and examined pathologically. The graft bones showed minimum new bone deposition with dysplastic epithelium. The epithelium was proliferative on the surface of graft bones forming epithelial strands and nests, similar to the odontogenic epithelium. The immunohistochemical study was performed using different antisera of odontogenic markers, growth factors, oncogenes, etc. The epithelial cells were strongly positive for pan-keratins, EGF, pAKT, and HSP-70, consistently positive for PCNA, p53, EGFR, 14–3–3, and survivin, slightly positive for ameloblastin, but rarely positive for amelogenin. Particularly the matrix of graft bone was slightly positive for EGF. Taken together, it is presumed that the abnormal epithelium on the graft bones was derived from odontogenic epithelial elements, Malassez epithelial rests, distributed at the periodontal tissue of maxillary molars, and that they might undergo dysplastic proliferation affected by the release of growth factors and osteogenic proteins from the graft bones. It is also suggested that the graft bone substitutes inserted for the dental implant possibly have a potential to induce the proliferation of odontogenic epithelial rests leading to the pathogenesis of odontogenic cysts and tumors.

Key words : Xenograft Bone, Odontogenic Epithelium, Dysplasia

1. INTRODUCTION

Different regenerative bone substitutes have been developed to play a role for the augmentation of extraction socket and alveolar ridge, and sinus floor elevation in order to prepare the appropriate bony site for dental implant¹. However, these bony substitutes inserted are expected to be resorbed and modified by new bone deposition, but some anorganic bovine bone is a non-resorbable bone substitute which can lead to stable bone over time².

Although there still exist a controversy whether the use of graft materials enhances new bone formation in contrast to natural healing alone³, the ridge preservation procedure using various graft materials is inevitable for the stabilization of dental implant⁴,⁵. As the autogenous, homogenous, and heterogenous bones can produce the
increased protein expression of RUNX2, BMP2, etc., the
different kinds of commercial graft bones are available
for the alveolar ridge augmentation and sinus floor
elevation.\(^6,7\)

Recently many authors have reported that the occur-
rence of oral squamous cell carcinoma were closely
associated with dental implant\(^9\), and that the cancer
cells were infiltrated into the alveolar bone of dental
implant, where regenerative bone graft materials were
inserted\(^9\). The fact that the cancer cells were intimately
adhered to the graft bones which contained abundant
BMP–2 might be related to the growth and invasion via
the signaling of transforming growth factor–β (TGF–β)
and chemokine (C–C motif) ligand 5\(^10\). Up to date
many cases of peri–implant primary tumors and peri–implant metastases have been also described, and
most of the primary tumors arisen from the peri–implant tissue are squamous cell carcinoma\(^11\).

The present study demonstrated a case of graft bone
biopsy exhibiting dysplastic proliferation of odontogenic
epithelium, which still did not produce any cystic lesion
or odontogenic tumor. With the results of immunohisto-
chemical (IHC) stains these abnormal epithelia were
further investigated for their origin and potential
propagation.

II. CASE REPORT

A 57 years old female received bone graft operation
for the extraction socket augmentation of right maxillary
molars and for sinus floor elevation using xenogenic
bone substitute (In–C, the company name not shown)
in a local dental clinic six months ago. The bone graft
sites were healed uneventfully and showed marked
radiopacity in the extraction sockets and the sinus
floor. Before dental implant insertion the bone biopsy
was made using trephine bur and examined
pathologically. The usage of biopsy specimens was
approved by the institutional review board of Gangneung–
Wonju National University Dental Hospital (IRB2013–2).

The removed specimen was decalcified with 5% nitric acid, routinely prepared for paraffin sections in 4 µm thickness, stained with hematoxylin and eosin (HE), and followed by the IHC stains using the triple sandwich indirect method described previously\(^12\).

Histologically the graft bone tissue showed minimum
new bone deposition with marked inflammatory reaction,
and also accompanied with the dysplastic proliferation
of epithelium on the graft bones (Fig. 1 A1). The
epithelium was actively proliferative on the graft bones
forming epithelial strands and nests, similar to the
odontogenic epithelium (Fig. 1 A2–5). However, the
epithelial cells were relatively well differentiated and
showed no feature of odontogenic tumor. At first glance
the tumorous epithelial growth was suspicious to be
originated from sinus mucosa, but the endoscope
observation showed no abnormality in the sinus mucosa.

The immunohistochemical study was subsequently
performed using different antisera of odontogenic markers
(amelogenin\(^\ast\) and ameloblastin\(^\ast\)), proliferating cell marker
(proliferating cell nuclear antigen (PCNA\(^\ast\)), cytokeratin
(pancytokeratins (pan–K\(^\ast\), cytokeratins No. 1, 2, 5, 6, 7,
8, 11, 14, 16, 17, 18)), growth factor (epithelial growth
factor (EGF\(^\ast\)) and epithelial growth factor receptor (EGFR\(^\ast\)),
protective protein (heat shock protein–70 (HSP–70\(^\ast\)),
and oncogenes (phosphorylated V–akt murine thymoma
viral oncogene homolog (pAKT\(^\ast\), phosphorylated at
Thr 308), 14–3–3\(^\ast\), survivin\(^\ast\), and p53\(^\ast\)). (\(^\ast\)Santa Cruz
Biotech., USA; \(^\ast\)NEOMARKERS, USA; \(^\ast\)DAKO, Denmark)

The abnormal epithelia found near the graft bones
were strongly positive for pan–keratins, EGF, pAKT,
and HSP–70, consistently positive for EGFR, 14–3–3, and
survivin, slightly positive for ameloblastin, but rarely
positive for amelogenin (Fig. 1 D–I and 2 A–C). The immunoreactions of PCNA and p53 were usually found in the cytoplasms of epithelial cells but rarely found in the nuclei of epithelial cells (Fig. 1 B and C). And the matrix of graft bone was slightly positive for EGF but negative for EGFR.

Generally the epithelium showed low level of cellular proliferation but its metabolic and oncogenic signaling were active, and underwent focally dysplastic proliferation. With the IHC evidences of ameloblastin positive but amelogenin negative\textsuperscript{13–15} the epithelium was potentially a derivative from primitive odontogenic epithelium.

The epithelium did not produce any cystic space, but it gradually covered the fibrous tissue with thin membranous structure, mimicking a lining epithelium of odontogenic cyst. However, except the histological findings the maxillary lesion was asymptomatic and the surgical wound was healed uneventfully. Thereby, the patient was appointed to be follow-up every 2 months, and so far there appeared no clinical and radiological changes for 6 months after this bone biopsy examination.

**IV. DISCUSSION**

The osteogenetic effect of different regenerative bone substitutes may contribute to preserve the atrophied alveolar ridge before dental implant insertion. Because the bony remodeling of graft bone may take a long period more than 3–6 months, the pathological examination of graft bone is seldom performed, if there appeared no clinical symptom. However, it has been known that the inserted graft bone particles are not
completely resorbed and remodeled by new bone replacement\textsuperscript{16,17}.

Even though the whole graft bones are not able to be examined in each sporadic reports of bone biopsy in the literature, it is considered that large portions of graft bones are still remained in the graft sites as an osteoconductive matrix or rigid mass stabilizing the bony defect\textsuperscript{18,19}. However, these graft bones may continuously release BMP proteins (TGF–β family) and eventually produce stromal fibrosis, and also contain different kinds of growth factors which can activate the nearby marrow tissues\textsuperscript{20,21}.

With the immunostains of ameloblastin and amelogenin the epithelium was identified to be derived from the primitive odontogenic epithelial rests in this study. The positive reactions of PCNA and p53 were localized only at the cytoplasms of epithelial cells, thus it is presumed that the epithelial cells were dysplastic rather than neoplastic in nature.

The epithelial cells were consistently positive for different oncogenic proteins including pAKT, 14–3–3, survivin, which are dysplastic change biomarkers of tumor cells, and more they were also positive for HSP–70, which is a biomarker of cellular protection against stressful environment. Therefore, it is presumed that the epithelial cells might underwent dysplastic proliferation in the graft tissue located at the periapical area of maxillary molars.

Particularly the dysplastic odontogenic epithelium was slightly positive for EGFR and strongly positive for EGF together with the slight reaction of EGF in the graft bone matrix. The coincident positive reaction of EGF both in the epithelium and the graft bone matrix might directly indicate that the abnormal proliferation of odontogenic epithelial rests was relevant to the graft bones. These molecular findings imply that the commercially available graft bones of allogenic or xenogenic origin may contain small amount of growth factors, which can affect the growth of dormant odontogenic epithelial rests.

Actually a case of postoperative maxillary cyst occurred 10 years after maxillary sinus augmentation using allogenic bone substitute was already reported,\textsuperscript{22} and it was also suggested that careful removal of pathologic apical lesion should be done before the insertion of regenerative bone substitute in the periodontal area\textsuperscript{23,24}. However, the present case showed no feature of odontogenic cyst/tumor development in the bone biopsy observation, and was asymptomatic during 6 months of follow-up. Further careful investigation will be followed.

Conclusively, with the results of IHC stains it is presumed that the abnormal epithelium on the graft bones was derived from odontogenic epithelial elements, Malassez epithelial rests, distributed at the periodontal tissue of maxillary molars, and that they might undergo dysplastic proliferation affected by the growth factors and osteogenic proteins released from the graft bones. It is also suggested that the graft bone substitutes inserted for the dental implant possibly have a potential to induce the proliferation of odontogenic epithelial rests leading to the pathogenesis of odontogenic cysts and tumors, and that careful bone biopsy be necessary to confirm the proper bony remodeling after the allogenic and xenogenic bone grafts.

V. REFERENCES


