Squamous Cell Carcinoma of the Oral Cavity in an Infant Cynomolagus Monkey

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Abstract: Squamous cell carcinoma was observed in the oral cavity in a one-year-old male cynomolgus monkey. Histopathologically, the tumor consisted of various shaped cells and its assemblies infiltrated into the surrounding connective tissues. Although no obvious metaplastic keratinized cancer pearls were found in the tumor cells, the intercellular bridges were observed. Immunohistochemically, tumor cells were stained with anti-keratin, but not with anti-vimentin. On virological examinations, no papilloma virus antigen or Epstein-Barr Virus small mRNA could not be detected. Under the electron microscope, incomplete tonofibrils and desmosomes in the cytoplasm and microvillus of the cell membrane were observed, suggesting a malignancy or low differentiation of the tumor cells in the present case. This is the first case of squamous cell carcinoma observed in very young macaques, to our knowledge.

Key word: cynomolgus monkey, infant, squamous cell carcinoma

Spontaneous squamous cell carcinoma is a type of neoplasm which frequently originates in the squamous epithelial cells in non-human primates such as several species of macaques, squirrel monkeys, marmosets, and tupaias [1, 3, 4]. The squamous cell carcinoma in macaques generally occurs in the oral cavity, esophagus, vagina and penis [1]. Furthermore, these cases are known to usually occur in adult or aged monkeys [1, 3, 4].

In the present case, however, squamous cell carcinoma in the oral cavity of a cynomolgus monkey (Macaca fascicularis) was found in a 1-year-old infant. Flaring of the gingiva in both the talon and maxilla, and puffiness of the left cheek and maxillary gingiva...
were noticed at 360 days after birth. The surrounding skin tissue in this area was normal at this stage. X-ray examination revealed a tumor mass in the left buccopharyngeal part, and fracture of the zygomatic bone was seen. Since the tumor mass continuously increased in size, a surgical operation for ablation of the mass was done 4 months after the tumor was found. And then, 80 days after the operation, this monkey was euthanatized because of poor prognosis. At necropsy, the tumor mass was found to have developed a further 3 cm in diameter and there was ostolytic infiltration into the skull. Other organs appeared to be normal except for metastasis to the adjoining lymph nodes.

The tumor mass and lymph nodes from the left oral cavity were fixed in 10% neutral buffered formalin and embedded in paraffin. Deparaffinized 4 µm sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Masson’s trichrome. In addition, some of them were immunostained by the streptavidin-biotin-complex method (LSAB, DAKO, Carpinteria, CA). Primary antibodies were a mouse antibody to bovine vimentin (anti-vimentin; DAKO, diluted at 1:100) and rabbit antibodies to bovine keratin (anti-keratin; DAKO, 1:250) and to bovine papilloma virus (anti-BPV; DAKO, 1:150). Secondary antibodies were biotinylated goat antibodies to mouse IgG and rabbit IgG (Kirkegaad & Perry Laboratory, Inc., Gaithersburg, MD, 1:100). These immunoreactions were visualized with 3,3-diaminobenzidine tetrahydrochloride (Sigma Chemical Company, St. Louis, MO) and a hydrogen peroxide solution. To detect Epstein-Barr Virus Encoded small mRNAs (EBER), in situ hybridization (ISH) was conducted with a peptide nucleic acid (PNA) probe for EBER (DAKO) and a PNA ISH detection kit (DAKO).

For electron microscopic examination, small pieces of the tumor mass were prefixed with 2% glutaraldehyde, postfixed with 1% osmium tetroxide, and embedded in epoxy resin (Epok 812, Oken Company, Tokyo, Japan). Ultrathin sections were double-stained with uranyl acetate and lead citrate and observed under an electron microscope (JEM-1200EX, JEOL, Tokyo, Japan).

Histopathological examination revealed an infiltrative growth of carcinoma cells with an arborescent formation between abundant connective tissues (Fig. 1-a). The tumor mass consisted of polygonal-shaped (high differentiated) cells in the central portion and pyramidal- or spindle-shaped (low differentiated) cells in the surrounding portion. Some polygonal cells formed incomplete intercellular bridges (Fig. 1-b). Both types of cells contained frequent mitotic figures. There were necrotic foci in the central portion of some assemblies but no obvious squamous metaplasia, which is generally called keratin pearls, was observed in the tumor assemblies. Most tumor cells were negative for PAS staining, whereas some necrotic foci had a positive reaction. Based on these findings, this tumor in an infant monkey was regarded as having originated in squamous cells in the gingiva of the oral cavity.

Immunohistochemically, the tumor cells showed positive reaction with an anti-keratin antibody, but reaction
intensity differed among tumor cells of various shapes (Fig. 2). Circumference spindle- or pyramidal-shaped cells showed a weakly positive reaction, whereas polygonal-shaped cells and necrotic foci in the center had an intense reaction. The tumor cells were negative for anti-vimentin and -BPV antibodies. Fibrocytes in the connective were positive for anti-vimentin antibody. ISH for EBV were negative in the tumor cells.

On electron microscopy, most of the tumor cells were found to be round in shape with low electron density (Fig. 3-a). Some of the tumor cells were spindle shape with high electron density indicating a degenerating or necrotic stage of the cells. The round tumor cells had well developed rough endoplasmic reticula in the cytoplasm. A small number of immature fibrils, probably defective tonofibrils, were scattered in the cytoplasm. The shrunken structures with high electron density were sparsely found on the cell membrane, and were considered to be incomplete desmosomes (Fig. 3-b). These structures were more prominent in polygonal tumor cells than in spindle tumor cells. Furthermore, a lot of microvilli on the cell membrane were more frequently observed in spindle cells than in round cells (Fig. 3-b). No virus particle was observed in the tumor under the electron microscope.

Since these histopathological, immunohistochemical and electron microscopic features suggested the tumor cells have characteristics of squamous cells [2] such as, 1) cell-shape with indicating squamous metaplasia, 2) the formation of intercellular bridges, 3) positive for anti-keratin antibody, and 4) the existence of tonofibril- and desmosome-like structures. On the other hand, some features different from those of typical squamous cell carcinoma were also found, which were 1) the absence of typical keratin cancer pearls, 2) different intensity of immunoreactivity to anti-keratin antibody among various shaped cells, 3) the absence of clear tonofibrils and desmosomes and 4) the appearance of microvilli in the low differentiated spindle cells. These events reflected the histological malignancy and poor prognosis of this tumor [2].

Some cases of squamous cell carcinoma in the oral cavity of young humans are closely related with viral infection such as EBV [6] and human papilloma virus [5]. In the present case, neither virus particles nor
antigens of papilloma virus and mRNA of EBV were detected. Nevertheless, more sensitive methods, such as a polymerase chain reaction with primers for papilloma virus or EBV, should be applied to check the possibility of viral infection.

References