



SHORT COMMUNICATION

Expression of ATP-binding cassette transporter in human salivary ducts

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Summary P-glycoprotein expression has been observed in normal tissues as well as malignant tumours and thus does not appear to be induced by anticancer drugs. Knowledge of the distribution of ATP-binding cassette (ABC) transporters other than P-glycoprotein in normal salivary tissue is essential for understanding the physiological secretion or excretion of potentially toxic substances. Here the expression of ABC transporters was studied immunohistochemically in normal salivary gland tissue from nine patients. In striated duct cells, staining was strong for P-glycoprotein, multidrug resistance-associated protein (MRP) 1, MRP 2/canalicular multispecific organic anion transporter (cMOAT), and lung resistance-related protein (LRP). The staining intensity of acinar and intercalated duct cells for MRP 1 expression was distinct from that for MRP2/cMOAT, but was similar to that for P-glycoprotein. LRP was observed as particles between the nuclear and luminal membranes in the cytoplasm of intercalated duct cells. The expression of ABC transporters suggests that numerous transporters other than those studied might be isolated from normal salivary tissues. These observations indicate that these ABC transporters may not arise from any previous contact with anticancer drugs but are expressed physiologically. The achieved drug resistance as well as the physiological secretory function of ABC transporters could contribute to the responsiveness to chemotherapy of malignant salivary tumours.

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ATP-binding cassette transporters are ATP-dependent membrane-binding proteins that transport materials in and out of cells.¹ P-glycoprotein, which is the product of MDR 1, confers resistance to various anticancer drugs. It is detectable in squamous-cell carcinomas of the head and neck, showing various levels of expression among different

tumours,² as well as in normal tissues³ (Thiebaut et al., 1987).⁴ P-glycoprotein has been localised in salivary ducts,² and its expression has been identified in striated duct cells, excretory duct cells and acinar demilunar cells of normal major and minor salivary glands.⁵ We have also shown high P-glycoprotein expression in ductal cells of human salivary malignant tumours.⁶ Therefore, P-glycoprotein may be physiologically involved in an important part of the transporter system in the salivary glands. However, the exact role of numerous other ATP-binding cassette transporters in the salivary glands, in which excretion can be mediated by various mechanisms, remains unclear.

Abbreviations: cMOAT, canalicular multispecific organic anion transporter; LRP, lung resistance-related protein; MDR 1, multi-drug resistance gene 1; MRP, multidrug resistance-associated protein

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We have now investigated immunohistologically the expression of MRP 1, MRP 2/cMOAT and LRP in human salivary duct cells. Nine patients (five females, four males; mean age, 59.1 years; range, 38–76 years) were selected from the Department of Oral & Maxillofacial Surgery at Matsumoto Dental University Hospital. All were systemically healthy. Salivary gland specimens were obtained by neck dissection from patients who consented and had not been treated with anticancer drugs. The specimens were immediately embedded in OCT compound. Immunohistochemical staining was performed as previously described.⁶ The primary antibodies were diluted in phosphate-buffered saline with 1% bovine serum albumin and included the following: (a) mouse monoclonal antihuman P-glycoprotein (JSB-1; Novacastra Laboratories Ltd., Newcastle, UK; dilution 1:50); (b) mouse monoclonal antihuman MRP 1 (MRP m5; Alexis Biochemicals; dilution 1:50); (c) mouse monoclonal antihuman MRP 2/cMOAT (M₂ III-6; Alexis Biochemicals; dilution 1:50); and (d) mouse monoclonal anti-human LRP (LRP-56, Alexis Biochemicals; dilution 1:50). The secondary antibodies were rabbit antimouse IgG (Dako, Glostrup, Denmark; dilution 1:200) and antimouse fluorescein isothiocyanate-conjugated antibody (DAKO; dilution 1:200). For immunofluorescence staining, the specimens were counterstained with propidium iodide solution (50 µg/ml). Fluorescence labelling was analysed by confocal microscopy (laser scanning microscope 510; Zeiss, Jena, Germany) and with an argon–krypton laser. WBV filters (Olympus, Tokyo) were used for confocal laser scanning.

Expression of ATP-binding cassette transporters was observed in human salivary duct cells. P-glycoprotein was found on the basolateral membrane and in the luminal membrane of striated duct cells, as well as in the membrane and cytoplasm of the terminal part of excretory duct cells, as reported by Uematsu et al.⁵ MRP 1 was detected in striated duct cells, the cytoplasm of serous acinar cells and the luminal membrane of excretory duct cells. Expression of MRP 1 was weaker than that of P-glycoprotein (Fig. 1). Large concentrations of MRP 2/cMOAT were detected in the basolateral membrane of all duct cells. Intense staining was identified particularly at the communicating junction among striated duct cells. LRP was present in the luminal membrane and the cytoplasm of intercalated and striated duct cells. In the cytoplasm, LRP was observed as particles between the nuclear and luminal membranes (Fig. 2).

We have investigated the immunoreactivity of a variety of ATP-binding cassette transporters, and compared differences in the reactivity, tissue

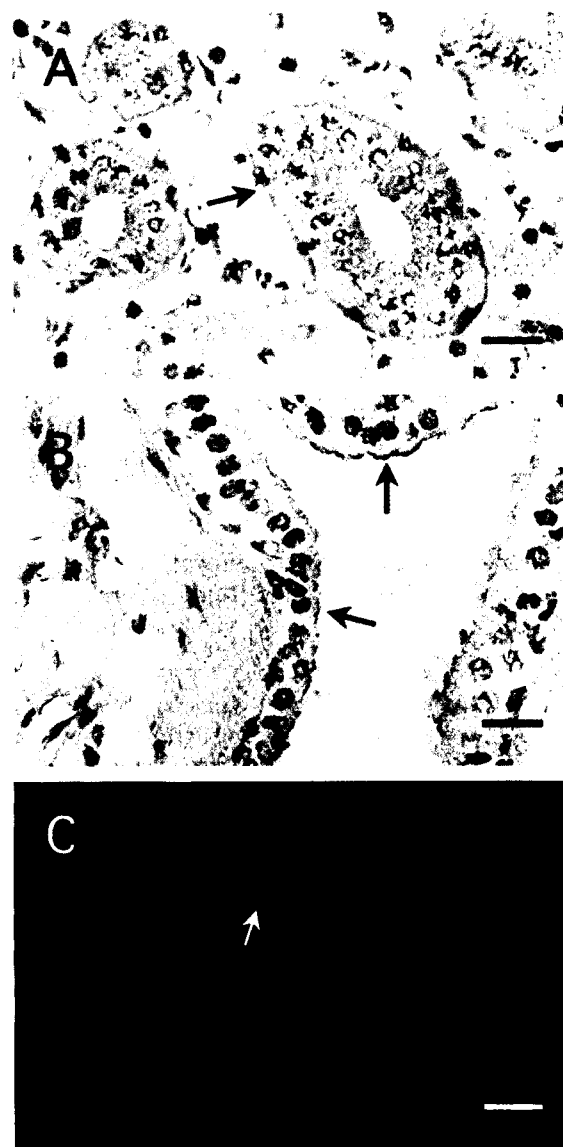


Figure 1 Expression of MRP 1. (A) Staining pattern of MRP 1 appears similar to that of P-glycoprotein (arrow). Bar = 10 µm. (B) Membrane staining at the terminal part of the excretory duct (arrows). Bar = 10 µm. (C) Weak staining of the striated duct cells by immunofluorescence labelling using fluorescein (arrow). Bar = 10 µm.

distribution and subcellular localisation of their expression with those previously described for P-glycoprotein. Expression of MRP 1, which transports the physiological substrate 17 α -glucuronosyl oestradiol,⁷ showed a similar distribution to that of P-glycoprotein. MRP 1 expression in the membranes of striated and excretory duct cells was higher in regions showing high ATPase activity. On the other hand, oestradiol, testosterone and cortisol, which are substrates of P-glycoprotein, are secreted in human saliva from blood.^{8,9} These findings suggest that P-glycoprotein and MRP 1 are localised in the

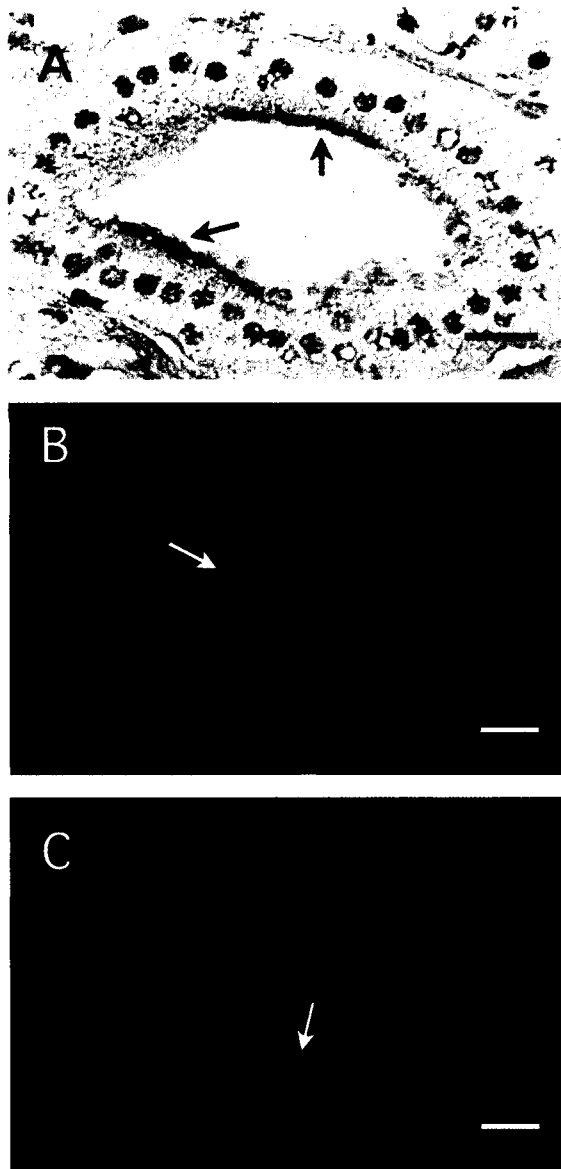


Figure 2 Expression of LRP. (A) Strong staining at luminal sites and near the nuclear membrane of the striated duct cells (arrows). Bar = 10 μm . (B) Expression is prevalent at basolateral sites and around the nuclear membrane (arrow) of the striated duct cells with fluorescence labelling using fluorescein. Bar = 10 μm . (C) LRP immunoreactivity appears as particles mainly in the apical region of the cytoplasm and near the nuclear membrane using confocal laser scanning imaging (arrow). Bar = 10 μm .

same tissues but transport different substrates. Indeed, Keppeler et al.⁷ report that the substrate specificity of MRP 1 is very different from that of MDR 1 P-glycoprotein.

MRP 1 and MRP 2/cMOAT are involved in excretion of organic anions from cells.¹⁰ MRP 2/cMOAT is localised in the apical membrane of the liver and kidney.¹¹ In the present study, MRP 2/cMOAT expression

was observed in the basolateral membrane of salivary duct cells. The differences in the location of MRP 2/cMOAT between the liver or kidney and the salivary duct might help clarify the role of organic anions and MRP 2/cMOAT in each organ, such as a barrier function, which would protect oral tissues from circulating toxic products in salivary duct cells. Alternatively, their functional organisation could serve a specific structural role, as would be evidenced if the functional interface was similar between the organic anions and cMOAT in these organs. LRP was weakly visualised as subcellular particles in the acini or striated duct cells. Immunoreactivity was observed only in or near the nuclear membrane, as noted by Chugani et al.,¹² and in the luminal cytoplasm. Although the role of LRP expression has recently attracted interest separately from MDR, LRP has been shown to have a transport function, acting as a carrier and mediating bidirectional nucleocytoplasmic exchange as well as the vesicular transport of compounds.^{12–17} The present results suggest that LRP plays a part in membrane trafficking rather than acting as an overexpressed transmembrane transporter that decreases intracellular drug accumulation. The physiological functions of LRP protein and the structural specificity of LRP substrates should also be examined. Therefore, the presence of these transporters in normal salivary gland tissue not previously exposed to anticancer drugs suggests they can occur physiologically in normal tissue, similar to the observed expression of P-glycoprotein in normal tissue demonstrated by van der Valk et al.⁴ and Uematsu et al.⁵

In general, chemotherapeutic agents or combination regimens for malignant salivary neoplasms produce inconsistent results and chemotherapy is administered only for palliation in symptomatic patients with recurrent and/or unresectable cancers.¹⁸ This could, in part, be explained by the finding that physiological and/or induced expression of ATP-binding cassette transporters other than the previously described P-glycoprotein, such as MRP 1, MRP 2/cMOAT, and LRP, occurs in human submandibular salivary glands. These transporters may be part of, and/or may contribute to, the active transportation of various substrates. Understanding the biology and transportation of cytotoxic or cytostatic agents that have therapeutic value is necessary for the control and treatment of malignant tumours of the salivary ducts.

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