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Short note

Intracellular localization of the matrix enzyme lysyl oxidase in polarized epithelial cells

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Abstract

Considerable evidence supports novel functions for lysyl oxidase (LOX) beyond its traditional role in initiating cross-linkages in collagen and elastin within the extracellular matrix. These novel roles are particularly relevant during the transition of malignant epithelial cells towards a migratory and invasive phenotype. However, knowledge on cellular and matrix functions of LOX has been generated almost exclusively in mesenchymal cell types. But it is becoming increasingly evident that these cell types are not adequate to address these novel and highly significant roles for LOX in epithelial tissues. In this initial report, we demonstrate that active LOX is expressed by polarized MDCK II kidney and MCF-10A breast epithelial cells. Furthermore, we show evidence for the presence of mature LOX in the cytoplasm and establish these cell lines as models for epithelial LOX studies.

Keywords: Lysyl oxidase (LOX); Epithelia; MDCK II cells; MCF-10A cells

1. Introduction

Lysyl oxidase (LOX) is essential for cross-linking of elastin fibers and collagen fibrils in extracellular matrices, thereby providing tissue integrity (reviewed in Kagan and Li, 2003; Fong et al., 2006). LOX function has been traditionally investigated using mesenchymal cell types as in vitro model systems, partially because those cells are believed to contribute most of the extracellular matrix components to the tissue stroma. Although epithelia face the extracellular matrix with their basal surfaces, there is poor knowledge about a potential role of LOX in epithelial cell types. A few reports, however, did provide evidence for LOX expression in some epithelial tissues based on immunohistochemistry data and one study detected LOX mRNA expression in cultured rabbit pigment epithelium cells (Omori et al., 2002; Hayashi et al., 2004; Noblesse et al., 2004; Fogelgren et al., 2005). Additionally, recent findings indicate that LOX is a critical factor when breast epithelial tumor cells acquire a migratory and invasive phenotype in vitro (Kirschmann et al., 1999, 2002; Payne et al., 2005). A recent study further demonstrated that LOX is essential for hypoxia-induced metastasis in a breast cancer model in vivo (Erler et al., 2006).

Altogether, these observations raise the fundamental question for a role of LOX in epithelial cells and, furthermore, how this function might be altered during the course of malignant progression in cancer. Using two well-characterized epithelial cell lines, MDCK II and MCF-10A cells, we show for the first time that active LOX is expressed by normal polarized epithelial cells in culture. We additionally provide evidence that LOX is not only secreted into the extracellular space where it is thought to be subsequently processed, but that processed LOX is also present in the cytoplasm of these cells. We suggest these cell lines might be appropriate to serve as valuable model systems for future in vitro studies that aim to understand the unexplored role of LOX in epithelia.

2. Results and discussion

2.1. LOX expression in polarized epithelial cells

Over the years, extensive studies have demonstrated that MDCK II and MCF-10A cells exhibit crucial features of polarized epithelia in vivo, most importantly apico-basolateral

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polarity (Yeaman et al., 1999; Debnath et al., 2003). Both cell lines display the characteristic "cobblestone" morphology of epithelial cells in culture (Fig. 1A).

It has been established that cultured epithelial cells in a pre-confluent stage reflect many properties of undifferentiated epithelia in vivo, whereas post-confluent monolayers possess key features of differentiated epithelia in vivo (Simons and Fuller, 1985). We first analysed LOX expression in pre-confluent and post-confluent cultures of MDCK II and MCF-10A cells. Initially, we examined LOX expression at the mRNA level and detected LOX transcripts by semiquantitative RT-PCR in each cell line (Fig. 1B). LOX mRNA levels seemed to be slightly elevated in post-confluent cells compared to those of pre-confluent cultures. To determine LOX protein expression, we subjected cell lysates as well as extracellular medium fractions to Western blot analysis. LOX is known to be synthesized as a glycosylated 50 kDa inactive pro-enzyme that is secreted into the extracellular space and subsequently processed by Bone Morphogenic Protein 1 (BMP-1) into the 30 kDa active form (Smith-Mungo and Kagan, 1998). We detected mature 30 kDa LOX in conditioned cell medium especially from post-confluent cultures in both cell lines (Fig. 1C), suggesting that cells in differentiated epithelial tissues might contribute to LOX-catalyzed cross-linking of ECM fibrils at their underlying basal surfaces and basement membranes. Surprisingly, we detected even higher amounts of 30 kDa LOX in cell lysates that were cleared from nuclei (Fig. 1C), indicating the presence of mature LOX in the cytoplasm of these cells. Interestingly, the amount of 30 kDa LOX in cell lysates is approximately equal within pre- and post-confluent cells, implying that intracellular LOX expression may be independent of the differentiation state in epithelial cells. Potential extracellular contamination in cell lysates seemed to be unlikely as fibronectin was exclusively detected in medium fractions (Fig. 1D). Consistent with these results is the presence of BMP-1 in both cell lysates and medium fractions of MDCK II and MCF-10A cells (Fig. 1E). Using immunofluorescence, we also observed intense cytoplasmic but not nuclear LOX staining in MDCK II cells, reinforcing our previous observations made by Western blot analysis (Fig. 2C, arrowheads indicate nuclei that were also counter-stained with DAPI, not shown).

We further investigated if mature LOX secreted by MDCK II and MCF-10A cells exhibits enzyme activity. We detected significant activity especially in medium fractions from post-confluent cultures of both cell lines (Fig. 2A, B). Notably, although on Western blots we observed the highest amounts of mature LOX in cytoplasmic fractions, we have not been able to detect lysyl oxidase enzyme activity from cell lysates yet (data

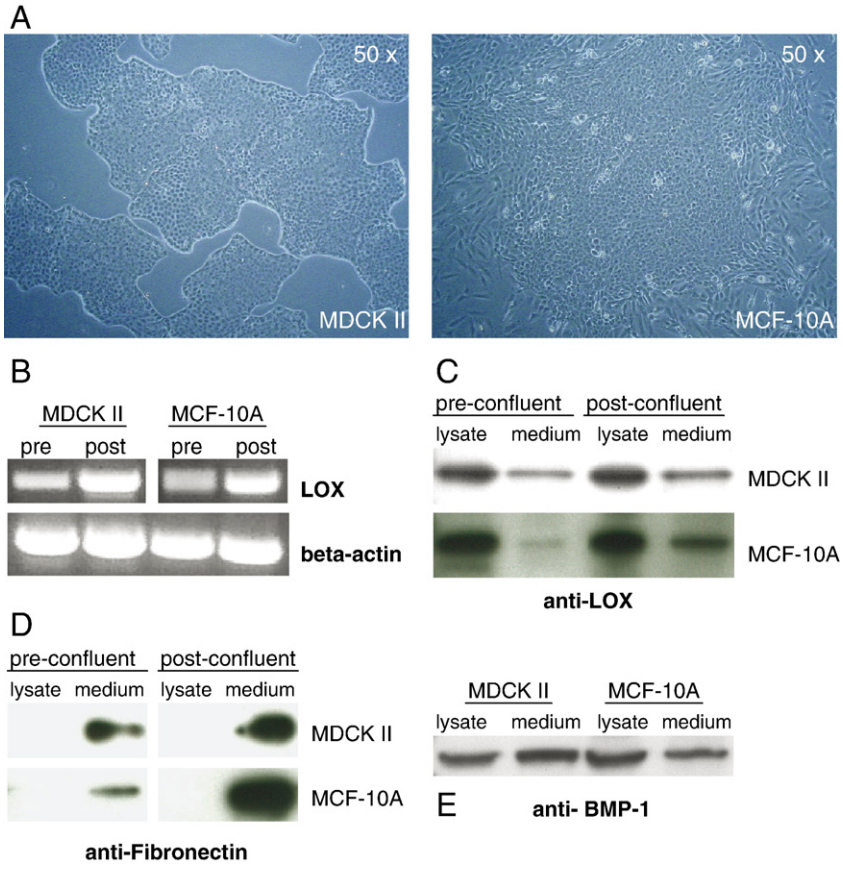

Fig. 1. Expression of lysyl oxidase (LOX) in polarized epithelial cells. (A) MDCK II cells and MCF-10A cells display the characteristic "cobblestone" morphology of polarized epithelial cells in culture. (B) Detection of LOX mRNA transcripts in MDCK II cells and MCF-10A cells by RT-PCR. Western blot analysis detected (C) mature (30 kDa) LOX protein in cell lysates and medium fractions, (D) fibronectin only in medium fractions and (E) mature (70 kDa) BMP-1 in cell lysates and medium fractions of MDCK II cells and MCF-10A cells.