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Aryl hydrocarbon receptor-dependent up-regulation of the heterodimeric amino acid transporter LAT1 (*SLC7A5*)/CD98hc (*SLC3A2*) by diesel exhaust particle extract in human bronchial epithelial cells

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ABSTRACT

The heterodimeric L-type amino acid transporter (LAT) 1/CD98hc is overexpressed in lung cancers with a poor prognosis factor. Factors that contribute to LAT1/CD98hc overexpression in lung cells remain however to be determined, but the implication of atmospheric pollution can be suspected. The present study was therefore designed to analyze the effects of diesel exhaust particle (DEP) extract (DEPe) on LAT1/CD98hc expression in bronchial epithelial BEAS-2B cells. Exposure to DEPe up-regulated LAT1 and CD98hc mRNA levels in a concentration-dependent manner, with DEPe EC_{50} values (around 0.2 μg/mL) relevant to environmental situations. DEPe concomitantly induced LAT1/CD98hc protein expression and LAT1-mediated leucine accumulation in BEAS-2B cells. Inhibition of the aryl hydrocarbon receptor (AhR) pathway through the use of a chemical AhR antagonist or the siRNA-mediated silencing of AhR expression was next found to prevent DEPe-mediated induction of LAT1/CD98hc, indicating that this regulation depends on AhR, known to be activated by major chemical DEP components like polycyclic aromatic hydrocarbons. DEPe exposure was finally shown to induce mRNA expression and activity of matrix metalloproteinase (MMP)-2 in BEAS-2B cells, in a CD98hc/focal adhesion kinase (FAK)/extracellular regulated kinase (ERK) manner, thus suggesting that DEPe-mediated induction of CD98hc triggers activation of the integrin/FAK/ERK signaling pathway known to be involved in MMP-2 regulation. Taken together, these data demonstrate that exposure to DEPe induces functional overexpression of the amino acid transporter LAT1/CD98hc in lung cells. Such a regulation may participate to pulmonary carcinogenic effects of DEPs, owing to the well-documented contribution of LAT1 and CD98hc to cancer development.

1. Introduction

L-type amino acid transporter (LAT) 1/CD98hc is a heterodimeric plasma membrane protein consisting of a light chain (LAT1, encoded by the *SLC7A5* gene) linked to a heavy chain (CD98hc, also known as 4F2hc, and encoded by the *SLC3A2* gene) by an extracellular disulfide bridge (Fotiadis et al., 2013). LAT1 is responsible for L-type sodium-independent uptake of large neutral amino acids such as leucine, isoleucine, methionine or tyrosine across cell membrane (Kanai et al., 1998). Molecular basis of this transport corresponds to an obligatory amino acid exchange with 1:1 stoichiometry (Verrey, 2003); the efflux substrate is in parallel handled by a sodium-dependent unidirectional influx system A or N amino acid transporter, that appears therefore to be the primary source of the driving force for LAT1-mediated transport. In addition to neutral amino acids, thyroid hormones as well as a few drugs like L-DOPA, alpha-methyldopa, melphalan, and gabapentin are transported by LAT1 (del Amo et al., 2008; Kinne et al., 2011). With respect to the heavy chain CD98hc, it serves as a chaperone for LAT1, allowing localizing and stabilizing it at the plasma membrane of cells, without transport function (Palacin and Kanai, 2004). CD98hc also plays this role towards other amino acid transporters to which it is covalently linked such as LAT2/*SLC7A8* and the L-type sodium-dependent amino acid transporters y^+LAT1/*SLC7A7* and y^+LAT2/*SLC7A6* (Fotiadis et al., 2013).

Amino acids transported by LAT1 serve not only for protein synthesis, but also as signaling molecules (Taylor, 2014). The essential amino acid leucine is thus a master regulator of mammalian target of rapamycin complex 1 (mTORC1) (Nicklin et al., 2009), a signaling pathway involved in promotion of cellular growth through phosphorylation of substrates that potentiate anabolic processes such as mRNA translation

Abbreviations: AhR, aryl hydrocarbon receptor; BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid; CYP, cytochrome P-450; DEP, diesel exhaust particle; DEPe, diesel exhaust particle extract; DMSO, dimethyl sulfoxide; EGR1, early growth response 1; ERK, extracellular regulated kinase; FAK, focal adhesion kinase; LAT, L-type amino acid transporter; MMP, metalloproteinase; mTORC1, mammalian target of rapamycin complex 1; p70S6K, p70S6 kinase; PAH, polycyclic aromatic hydrocarbon; PI3K, phosphatidylinositol-3 kinase.

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and lipid synthesis, or limit catabolic processes such as autophagy (Laplante and Sabatini, 2012). By this way, LAT1 is thought to favor cancer cell proliferation and metastasis (Fuchs and Bode, 2005; Wang and Holst, 2015). CD98hc also independently promotes cancer development, through its direct binding to β-integrins (mainly β1 but also β3), which is presumed to modulate tumorigenic integrin-dependent events such as survival, proliferation, migration and transformation (Feral et al., 2005). As an example, CD98hc has been shown to control activity of focal adhesion kinase (FAK), an integrin-related signaling way, which in turn may result in modulation of the matrix metalloproteinase (MMP)-2 (Santiago-Gomez et al., 2013), strongly implicated in cancer progression (Bauvois, 2012).

Both LAT1 and CD98hc have been shown to be overexpressed in many cancers (Kaira et al., 2008). They are notably detected in non-small cell lung cancers, for which they are considered as poor prognosis parameters (Imai et al., 2009; Kaira et al., 2010; Fei et al., 2014). Regulatory factors that may be involved in the induction of their expression in lung cells remain however to be characterized. A role for atmospheric pollutants may be suspected, notably for diesel exhaust particles (DEPs), which are now recognized as carcinogenic for the lung (Vermeulen et al., 2014). Indeed, DEPs, originating from diesel engines and to which humans are widely exposed in industrialized and/or urban areas (Gouge et al., 2010), are composed of a center core of elemental carbon to which various chemicals, especially polycyclic aromatic hydrocarbons (PAHs), are adsorbed (Wichmann, 2007) and such PAHs are potent activators of the aryl hydrocarbon receptor (AhR) signaling pathway (Puga et al., 2009), involved in LAT1 mRNA up-regulation in hepatocytes (Sarkar et al., 1999; Le Vee et al., 2010). The present study was therefore designed to determine whether exposure to DEP extract (DEPe) can regulate LAT1/CD98hc in human bronchial epithelial BEAS-2B cells. Our results indicate that DEPe induces expression of both LAT1 and CD98hc in an AhR-dependent manner in lung BEAS-2B cells. DEPe is also demonstrated to concomitantly increase uptake of leucine and to up-regulate MMP-2 in a CD98hc-dependent manner, thus bringing evidence that DEPe-mediated up-regulation of LAT1/CD98hc has functional consequences in lung cells.

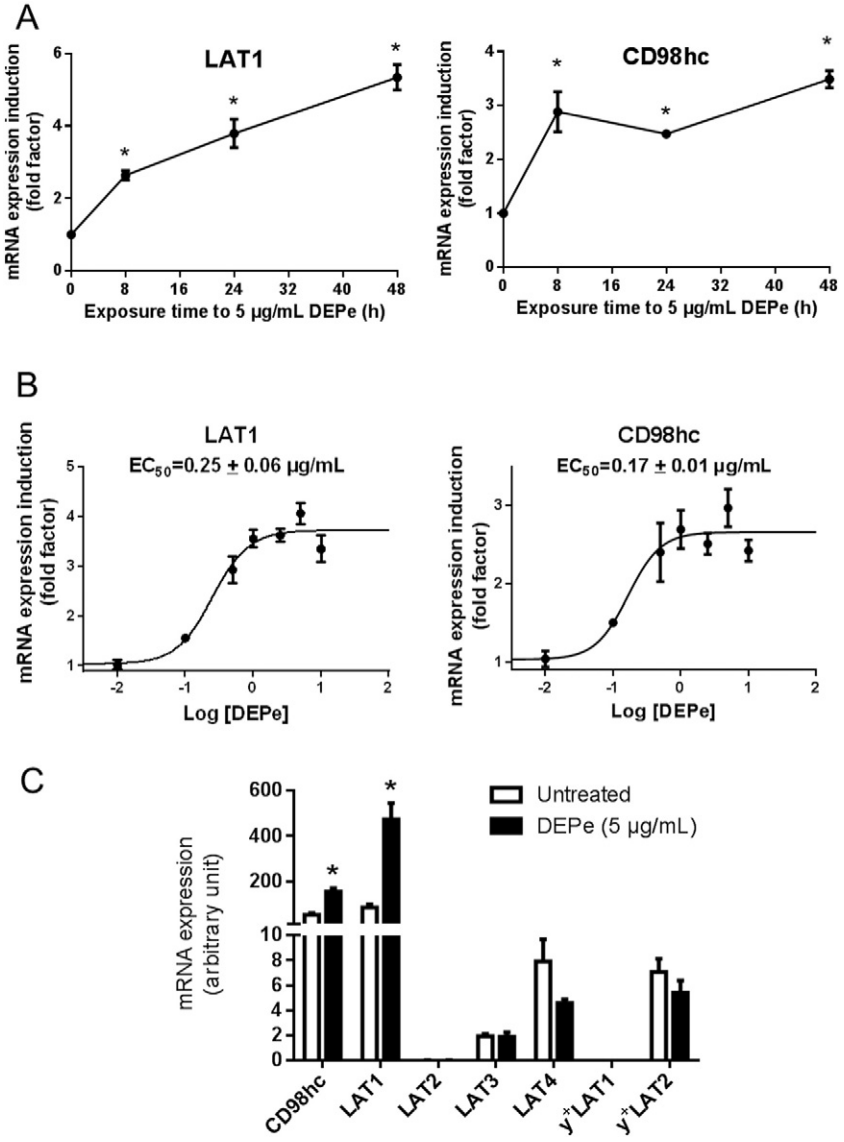

Fig. 1. Induction of LAT1/CD98hc mRNA expression by DEPe. BEAS-2B cells were either untreated or exposed to (A) 5 µg/mL DEPe for various lengths of time (from 8 h to 48 h), (B) to various DEPe concentrations (from 0.01 to 10 µg/mL) for 24 h or (C) to 5 µg/mL DEPe for 24 h. (A, B) LAT1, CD98hc and (C) other amino acid transporter subunit mRNA expressions were next determined by RT-qPCR. Data are expressed (A, B) as fold induction factor comparatively to mRNA levels found in untreated cells or (C) in arbitrary units, as described in Materials and Methods; they are the means ± SEM of three independent assays. (A, C) *, $p < 0.05$ when compared to untreated cells.