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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

RESEARCH LETTER

Mutant KRAS Exosomes Alter the Metabolic State of Recipient Colonic Epithelial Cells

See editorial on page 647.

In colorectal cancer (CRC) cells, mutant Kirsten rat sarcoma (KRAS) cell-autonomously imparts Warburg-like[1] metabolic changes through induction of Glucose transporter 1 (GLUT-1) (SLC2A1).[2,3] We previously reported that mutant KRAS has marked effects on the constituents of CRC exosomes, including proteins and enzymes involved in metabolism and glycolysis.[4,5] The present studies were designed to test whether mutant KRAS exosomes can alter the metabolic state cell-nonautonomously in recipient colonic epithelial cells.

We isolated exosomes purified from Daniel L. Dexter derived 1 (DLD-1) cells, which contain 1 wild-type (WT) and 1 mutant *KRAS* allele, and those from DLD-1 isogenic cell variants genetically engineered to express only the WT *KRAS* allele (DKs-8) or only the mutant *KRAS* allele (DKO-1).[6] Adding DKO-1 exosomes to DKs-8 cells significantly reduced glucose concentrations in the medium, suggesting increased cellular glucose uptake in these WT KRAS cells (Figure 1*A*). After 48-hour exposure to DKO-1 exosomes, a significantly higher percentage of recipient DKs-8 cells were in S and G2/M phases of the cell cycle (Supplementary Figure 1*A* and *B*), and cell number was increased at 120 hours (Supplementary Figure 1*C*). We next used the endogenous fluorescent characteristics of Nicotinamide adenine dinucleotide reduced (NADH) and Flavin adenine dinucleotide (FAD) to determine the relative balance between glycolysis and oxidative phosphorylation, as previously reported.[7–9] Addition of mutant KRAS exosomes selectively and significantly increased

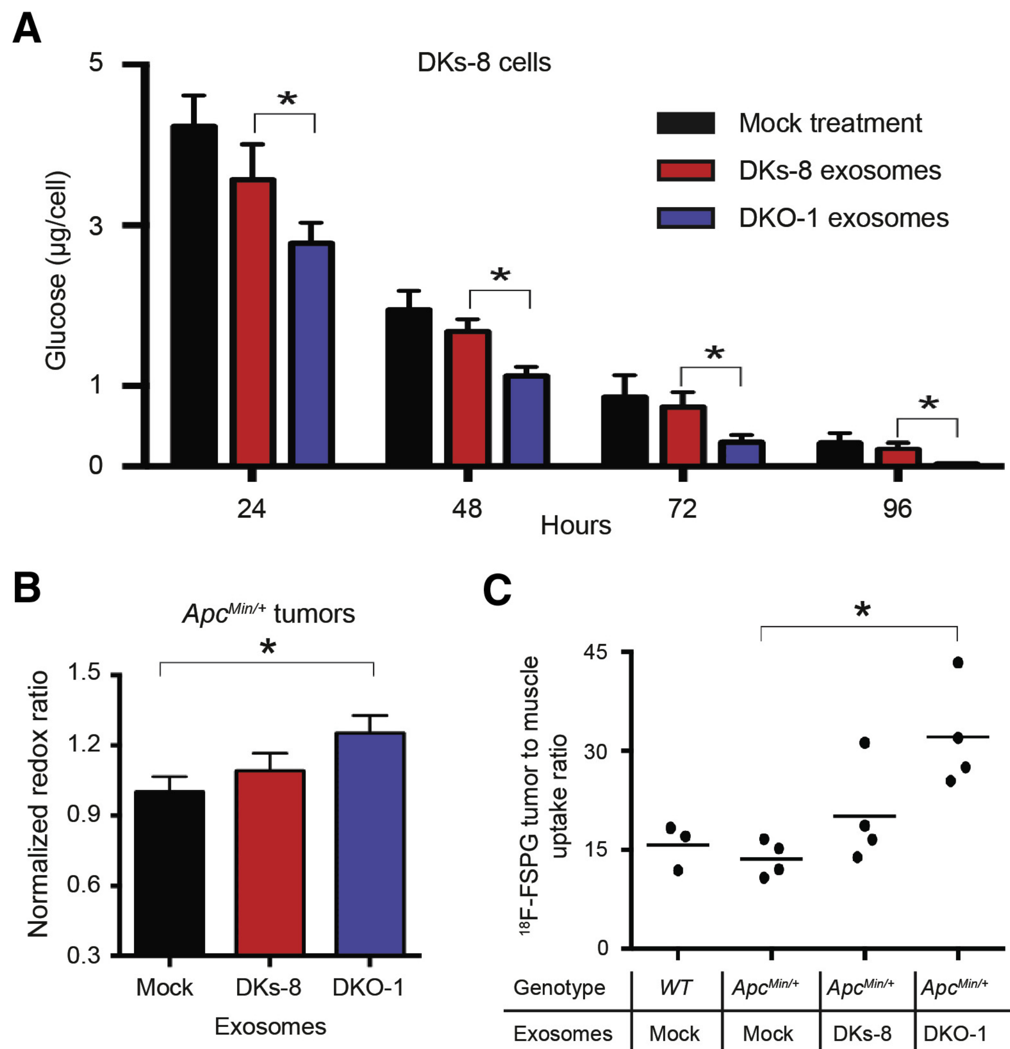

Figure 1. Mutant KRAS exosomes alter metabolism in vitro and in vivo. (*A*) Glucose consumption of exosome-treated DKs-8 cells (N = 3 in triplicate). Data are plotted as means ± SD. (*B*) Normalized redox ratio of exosome-treated $Apc^{Min/+}$ colonic tumors. (*C*) ^{18}FSPG tumor-to-muscle uptake ratio in WT or $Apc^{Min/+}$ mice injected with exosomes analyzed by 1-way analysis of variance followed by a post hoc Tukey test. *$P < .05$.

the redox ratio in recipient normal mouse colonic cells cultured in Matrigel or on plastic (Supplementary Figure 2*A*–*D*) and WT KRAS DKs-8 cells (Supplementary Figure 2*E*).

To test whether mutant KRAS CRC exosomes function in vivo, we used the Adenomatous polyposis coli multiple intestinal neoplasia ($Apc^{Min/+}$) mouse model in which adenomas develop throughout the gastrointestinal tract. $Apc^{Min/+}$ mice received intraperitoneal injections of DKs-8 or DKO-1 exosomes over 4 successive days. The redox ratio of tumors treated with DKO-1 exosomes was increased significantly (Figure 1*B* and Supplementary Figure 3*A*), suggesting that treatment with these mutant KRAS exosomes increases aerobic glycolysis in recipient tumor cells. We also performed (*S*)-4-(3-[^{18}F]-fluoropropyl)-*L*-glutamic acid (^{18}F-FSPG) positron emission tomography imaging 2 hours after the last injection of exosomes. ^{18}F-FSPG is a novel positron emission tomography tracer that follows the import of cystine by the glutamate/cysteine antiporter *SLC7A11*, which is overexpressed in CRC.[10] We found that $Apc^{Min/+}$ mice injected with DKO-1 exosomes had a significant increase in ^{18}F-FSPG uptake in the tumor region (Figure 1*C* and Supplementary Figure 3*B* and *C*), suggesting that mutant KRAS CRC exosomes can alter tumor cell metabolism in vivo.

Levels of GLUT-1 were increased in DKO-1 exosomes, as well as in cell lysates (Figure 2*A*). To test whether these mutant KRAS exosomes contained functional GLUT-1, we measured ^{18}F-fluorodeoxyglucose incorporation. After 1 hour, ^{18}F-fluorodeoxyglucose uptake was significantly higher in DKO-1 exosomes (Figure 2*B*). The purified exosomes showed the characteristic cup-shaped morphology and size reported for exosomes (40–100 nm) (Supplementary Figure 4*A* and *B*). Further purification of exosomes on an

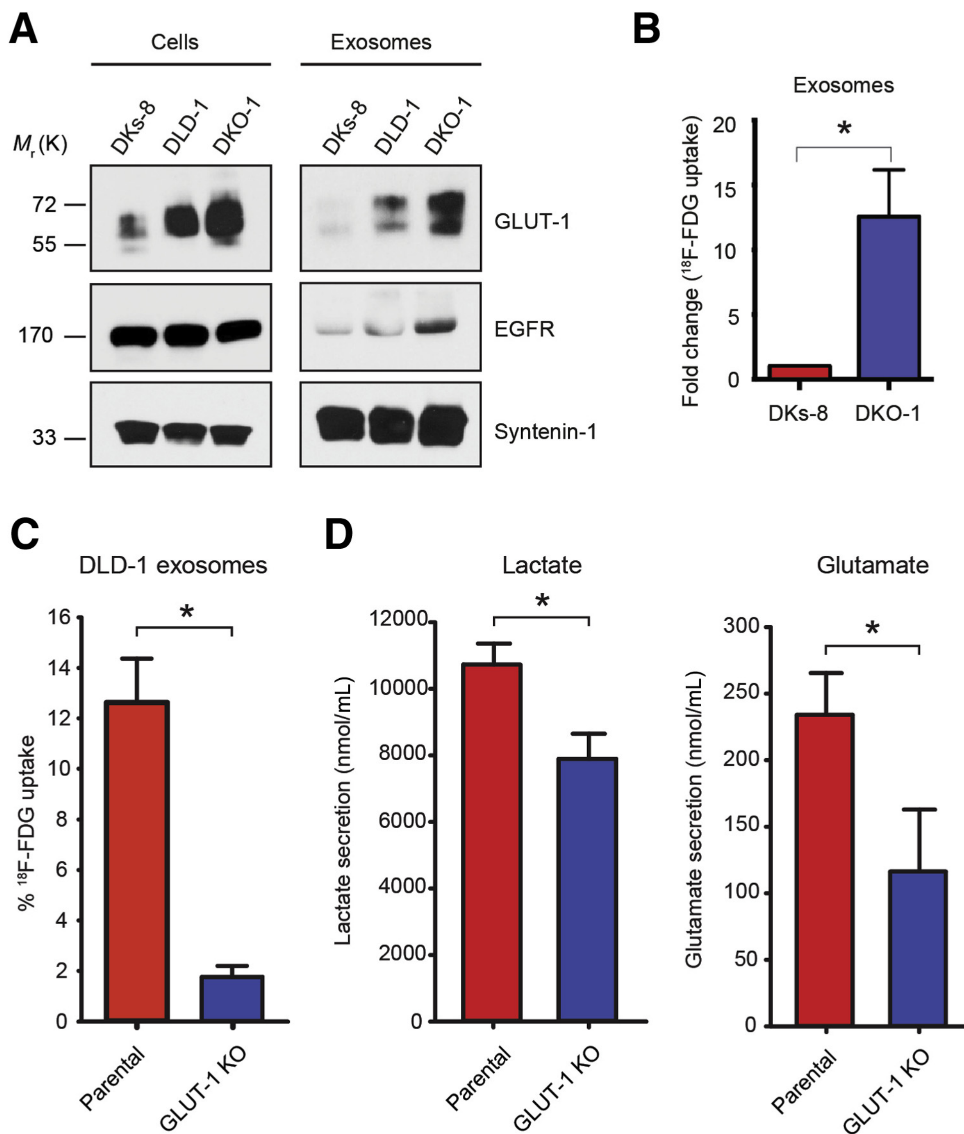

Figure 2. Exosomal GLUT-1 partially drives metabolic changes in recipient cells. (*A*) Immunoblot analysis of cells and exosomes. After normalization to syntenin-1, levels of GLUT-1 were increased 2.5- and 3.1-fold in cell lysates and 3.1- and 5.2-fold in exosomes of DLD-1 and DKO-1 cells, respectively. (*B*) Percent ^{18}F-fluorodeoxyglucose (^{18}FDG) uptake in exosomes isolated from DKs-8 and DKO-1 cells (N = 3 in triplicate). (*C*) Fold-change of ^{18}FDG uptake in exosomes isolated from parental and GLUT-1 KO DLD-1 cells. (*D*) nuclear magnetic resonance (NMR) determination of glutamate and lactate secretion in recipient DLD-1 GLUT-1 KO cells 43 hours after treatment with exosomes from parental DLD-1 or GLUT-1 KO cells (N = 2 in triplicate). Data are plotted as the means ± SD. $^{*}P < .05$.

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