Limits to growth: Scientists identify key metastasis-enabling enzyme

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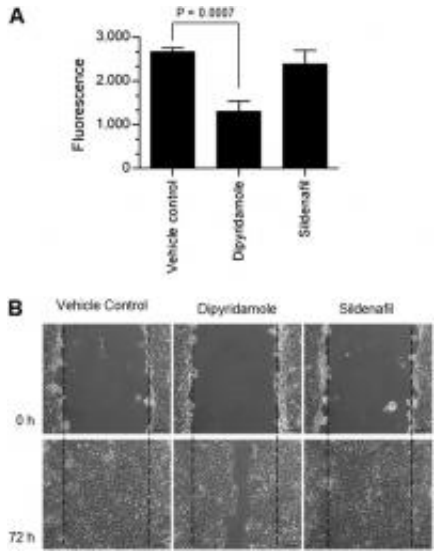

Inhibiting EDI3 glycerophosphodiesterase activity decreased migration in MCF-7 cells. (A) 100 μg of MCF-7 whole cell lysates were preincubated for 10 min with 25 μM of either Dipyridamole or Sildenafil, before incubation with GPC for 60 min at 37 °C. Enzyme-coupled spectrophotometric assay showing choline production from GPC for both inhibitors and the vehicle control (DMSO). Bar graphs show mean ± SD of three independent experiments. Student t test was used for statistical analysis. (B) Representative images of MCF-7 scratch assay for cells treated with the vehicle control (DMSO) or 25 μM Dipyridamole or Sildenafil until wound closure in the control cells at 72 h. The experiment was performed three independent times. Images were taken using a 10× objective. Copyright © PNAS, doi: 10.1073/pnas.1117654109

(Medical Xpress) -- On the complex road to eradicating cancer, controlling or preventing metastatic growth initiated by primary tumors is high on the to-do list. A key area of such research is the development of therapies based on identifying markers of metastasis associated with altered choline metabolism in breast, ovarian, and prostate cancers. Recently, scientists at the Leibniz Research Centre for Working Environment and Human Factors (IfADO), University of Dortmund, Germany, studying the tumor metabolome – the characteristic metabolic phenotype of tumor cells fundamental to the tumor's metastatic capacity – identified EDI3 (endometrial differential 3) as the enzyme responsible for a decreased glycerophosphocholine (GPC) to phosphocholine (PC) ratio by cleaving GPC to produce choline. The scientists concluded that since inhibiting EDI3 activity corrects the GPC/PC ratio and thereby decreases tumor cell migration capacity, it represents a possible therapeutic modality.

Not surprisingly, Dr. Jan G. Hengstler and his team – Dr. Joanna D. Stewart, Dr. Rosemarie Marchan, Dr. Michaela S. Lesjak, and other researchers – had to deal with a number of challenges in identifying EDI3 as the critical enzyme in glycerophosphocholine cleaving. "The story started," recalls Hengstler, "when we scraped out a band from a silver gel. This band contained a gene expressed in metastasizing, but not non-metastasizing endometrial carcinomas. Nothing was known about the function of this gene, which we named EDI3 because its precursors EDI1 and EDI2 were not confirmed in independent clinical samples, and therefore not further studied." In fact, at that point EDI3 was not yet included on the Affymetrix genomic analysis chip, which may explain why EDI3 was completely unexplored.

"Some initial attempts to understand EDI3's function and relevance failed," Hengstler tells *Medical Xpress*. "At this time the small EDI3-project was almost dead. Fortunately, persistence and some brilliant ideas from two post docs and a PhD student - Rosemarie

Marchan, Joanna Stewart and Michaela Lesjak – gave the project new life. By some clever *in silico* studies, they came up with a small number of hypotheses on the mode of action of EDI3 – and one of them could indeed be experimentally confirmed." EDI3 cleaves glycerophosphocholine to release choline and glycerol-3-phosphate – an important metabolic step, because choline metabolism not only provides membrane phospholipids essential for neoplastic cells, but is essential for the activation of a number of signaling proteins. "This reaction, also known as Kennedy-pathway, was already in the textbooks, Hengstler adds, "and some components of the choline metabolism were even being explored as possible targets in cancer. However, a key enzymatic protein positioned at the start of this pathway remained unidentified."

As soon as the enzymatic mechanism was clear, the project progressed rapidly. "Through what I'd consider amazing teamwork," says Hengstler, "Marchan, Stewart and Lesjak worked together to establish methods that allowed them to manipulate EDI3's levels, while at the same time developing the reagents and methods needed to measure EDI3." The latter included the development of an EDI3 antibody and an enzymatic assay to measure EDI3's activity. "From this we learned that EDI3 has a tremendous influence on lipid patterns, particularly both lysophosphatidic acid and phosphatidic acid. Things got even more exciting when it became clear that phosphatidic acid creates membrane anchoring points for proteins that activate many intracellular signaling pathways, many that are altered in cancer. In addition, phosphatidic acid is a direct precursor to another important signaling lipid – diacylglycerol, which directly activates protein kinase C (PKC)." Activated PKC increases migration activity of several tumor cell lines, and increased migration contributes to the high metastatic capacity observed in EDI3 overexpressing carcinomas.

Further enhancements are already being implemented. "Currently, we're analyzing EDI3 to determine if it's also important for other cell functions

besides migration," Hengstler explains. "Ongoing experiments suggest that EDI3 does not influence proliferation but it seems to support tumor cell attachment and spreading – two further key steps in the metastasis process. Therefore, the key question is: does inhibition of EDI3 inhibit metastasis? Is EDI3 a good therapeutic target for drug development?"

Moreover, the team has defined the next steps in their research. "First," notes Hengstler, "we have to establish specific inhibitors with good pharmacokinetic properties, because the inhibitor we used in our previous work is rather promiscuous with other effects. For this purpose, we are currently collaborating with colleagues at the neighboring Max Planck Institute to screen a compound bank for inhibitors using purified EDI3 protein." Once isolated, the best inhibitors will then be tested for antimetastatic activity in mouse models.

Hengstler also stresses that although EDI3 may be used within a battery of several biomarkers, this will not be its hottest future research topic. "Today, biomarkers are better identified from genome, proteome or metabolome studies to systematically establish signatures that represent certain biological motifs relevant for prognosis," Hengstler explains. "Our group sees EDI3's future rather as therapeutic target and as a physiological master switch that links lipid metabolism to signaling network activities – and it should be considered that currently, a lot of energy is invested in studying choline kinase for tumor therapy. Choline kinase is a downstream factor of EDI3, and our data suggest that for some purposes, inhibiting EDI3 may be much more efficient."

Last but not least, says Hengstler, many of the lipids that are influenced by EDI3 create anchoring points for signaling molecules in the cell membrane. Relatively little is known how lipid metabolism is linked to the activity of signaling networks, and activation of signaling pathways often starts with recruitment of cytoplasmic signaling factors to lipid anchors in the cell membrane. "In our experiments," Hengstler points

out, “we identified an influence of EDI3 only on PKC signaling. Nevertheless, this influence may be cell type dependent.” EDI3 may therefore represent a much broader principle linking choline metabolism and signal transduction.

Hengstler also sees other areas of research, diagnosis and treatment potentially benefitting from the team’s findings. “Our focus on tumor research should not cloud our view of other areas where EDI3 might play a central role. One of our key findings was that EDI3 strongly influences intracellular lysophosphatidic acid, or LPA, which plays a critical role in many biological processes – for example, neuroregeneration, which leads to the question of why EDI3 is so strongly expressed in neurons. LPA is also reported to be responsible for the extreme itching experienced by many patients suffering from some liver diseases. Is EDI3 also relevant in this context? The discovery of EDI3 as a key enzyme in choline metabolism,” Hengstler concludes, “has definitely led to more questions than answers.”

More information: *Choline-releasing glycerophosphodiesterase EDI3 drives tumor cell migration and metastasis*, *PNAS*, Published online before print May 8, 2012, doi: 10.1073/pnas.1117654109