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PFIZER RESEARCH AWARDS 2024

CLINICAL RESEARCH

Syndecan-4 is a key maestro of stomach cancer progression with patient's prognosis association

Ana Magalhães [1,2]

[1] i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto 4200-135, Portugal.

[2] ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto 4050-313, Portugal.

✉ amagalhaes@i3s.up.pt

Scientific Background

Stomach cancer is often a silent disease, frequently diagnosed at advanced disease stage, and with still limited therapeutic options. Our research group and several others have shown that gastric cancer cells display aberrant cell surface glycosylation signatures, which hold promise not only as diagnosis biomarkers but also as novel clinical targets.

In recent years, a growing amount of scientific evidence has highlighted that protein post-translation modifications, such as glycosylation, harbour significant relevance and functional impact in determining cancer cell biological behaviour. The glycoconjugates (molecules modified with covalently linked carbohydrate chains) expressed at every cell surface glycocalyx are essential players within the tumour microenvironment. These glycosylated molecules display pivotal roles in defining extracellular matrix (ECM) biochemical and biophysical properties and shaping cancer cell communication in the tumour but also at distant sites. Among these molecular regulators, heparan sulfate proteoglycans (HSPGs), particularly Syndecan-4 (SDC4), are emerging as critical players in tumour development, cell migration, invasion, and extracellular communication [1-3].

Syndecans (SDCs) comprise a family of four type-I transmembrane HSPGs. SDCs are highly abundant at the cell surface and act in cooperation with different transmembrane receptors and ECM molecules with multiple functions in cell signalling, adhesion, proliferation, migration, apoptosis, and differentiation. The SDCs expression profile, and their glycosylation features, have been frequently described as aberrantly altered in various cancers, including gastrointestinal tumours. More-

over, we have recently reported that the heparan sulfate (HS) cellular balance shapes cancer cell motility features and contributes to gastric cancer cells aggressiveness [2].

Lately, proteoglycans have been pinpointed as important partners in extracellular vesicle (EVs) mediated communication in the tumour microenvironment. Particularly, SDCs have been implied in EVs biogenesis, cargo selection and secretion, as well as for defining cancer cell-derived EVs uptake by recipient cells, ultimately fine-tuning tumour dissemination.

Research Project

In this study we evaluated syndecan-4 (SDC4) expression in a retrospective series of 152 gastric carcinomas from Instituto Português de Oncologia do Porto (IPO-Porto). We showed that SDC4 is highly expressed in intestinal subtype gastric tumours and its expression significantly associates with lower patient overall survival. Noteworthy, a similar SDC4 expression profile was also observed in lymph node metastasis from the same patients. The independent prognostic value of SDC4 in the intestinal subtype gastric cancer was demonstrated by multivariate analysis.

To determine the cellular functional impact of SDC4, its expression was knock-out (KO) in the MKN74 intestinal subtype gastric cancer cell line using gene editing by CRISPR/Cas9. We evaluated the impact of SDC4 KO in cellular features by performing migration and invasion assays. We demonstrated that SDC4 promoted an aggressive cancer phenotype, characterized by higher cellular motility in different ECM contexts and increased invasion capacity.

We further addressed the impact of SDC4 KO on gastric cancer cells' EV secretion and their biological activity using *in vitro* assays and mice models. We provided evidence, for the first time, that fully glycosylated-SDC4 is packed on gastric cancer cells secreted EVs and impacts EV protein cargo, uptake by recipient cells and, importantly, defines the tropism of cancer EVs. Interestingly, lack of SDC4 led to the production of a higher amount of EVs, but with smaller size and a distinct protein cargo. On the other hand, the SDC4 on EVs associated with the presence of cancer-associated molecules, such as Transforming growth factor beta-1 proteins (TGFβ1) and growth arrest specific protein 6 (GAS6). We demonstrated that SDC4 is EVs is critical for EV uptake by recipient cells and that SDC4 and HS on EVs modulates their functional effects on the recipient cells, namely the invasion capacity. Noteworthy, mass spectrometry analysis of SDC4 KO cells treated with SDC4-positive EVs showed that these cells, with restored invasion capacity, presented de novo incorporation of SDC4, further supporting the SDC4-associated aggressive phenotype.

By organ distribution analysis of fluorescently labelled EVs in NOD SCID mice, we disclosed that SDC4 dictates the tropism of EVs for common gastric cancer metastatic sites, like the liver and lungs. Moreover, loss of SDC4 alters the EV protein cargo and reduces EV uptake by recipient cells, disrupting cancer cell communication.

Collectively, our findings highlight SDC4 as an independent prognostic factor for intestinal subtype gastric cancer, emphasizing its potential as a biomarker for aggressive disease. Furthermore, we disclose previously unappreciated roles of HS glycosaminoglycans in gastric cancer biology and unveil their potential as tumour biomarkers but also as therapeutic targets to block cancer cell signalling and communication [4].

Implications for Future Research and Clinical Practice

SDC4 emerges as a central regulator in gastric cancer progression and therefore as a promising target for therapeutic intervention. Our study suggests further investigation into the molecular mechanisms through which SDC4 influences EV-mediated communication and metastatic site tropism. Moreover, the molecular interaction of SDC4 with molecules like TGFβ1 and GAS6 in immune regulation within the tumour microenvironment remains to be further elucidated.

The association of SDC4 expression with patients' prognosis, supports that measuring SDC4 levels may help to identify patients at risk of aggressive disease progression. Moreover, proteoglycans in EVs have already been identified as relevant biomarkers for minimally invasive cancer diagnosis.

The understanding of SDC4 role in EV-mediated cell communication offers new insights into cancer metastasis and highlights its potential for advancing personalized oncology strategies targeting cancer dissemination. From a therapeutic perspective, blocking SDC4 or specific glycosylation features of SDC4 has potential to inhibit cancer cell invasion and prevent the establishment of metastatic niches.

Finally, our results support investment in the designing of specific inhibitors targeting HS biosynthetic enzymes and HS-protein interactions, including those mediated by SDC4, due to their potential as novel, still under explored, therapeutic approaches in cancer clinical settings.

PAPER DISCUSSED

Poças J, Marques C, Gomes C, et al. Syndecan-4 is a maestro of gastric cancer cell invasion and communication that underscores poor survival. *Proc Natl Acad Sci U S A. 2023;120(20):e2214853120.* doi:10.1073/pnas.2214853120

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