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Investigação sobre a ocorrência de reprogramação fetal no desenvolvimento do pâncreas endócrino em modelo animal de diabetes *mellitus* tipo 1

Dissertação apresentada ao Programa de Pós-graduação em Biologia de Sistemas do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Mestre em Ciências.

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Área de concentração: Biologia de Sistemas

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RESUMO

DIAS, C. Investigação sobre a ocorrência de reprogramação fetal no desenvolvimento do pâncreas endócrino em modelo animal de diabetes *mellitus* tipo 1 [dissertação (Mestrado em Biologia de Sistemas)]. São Paulo: Instituto de Ciências Biomédicas; Universidade de São Paulo, São Paulo; 2019.

Várias evidências, incluindo as originadas de estudos anteriores do LBR&MEC, sugerem que condições adversas durante o desenvolvimento intrauterino promovam alterações moleculares e estruturais em órgãos e sistemas vitais podendo comprometer o seu funcionamento no indivíduo adulto. A hiperglicemia é um fator que influencia negativamente o desenvolvimento fetal, modificando processos biológicos importantes, como o padrão de síntese e deposição dos componentes da matriz extracelular (MEC). A MEC participa diretamente do processo de ramificação e morfogênse do pâncreas, e pouco é conhecido a respeito dos efeitos da hiperglicemia materna sobre a MEC desse órgão durante seu desenvolvimento. Investigamos por meio de imuno-histoquímica como a hiperglicemia materna severa modifica a distribuição de panlaminina, das cadeias α1 eγ1 das lamininas e da integrina α3, moléculas da MEC que desempenham um papel chave na diferenciação do pâncreas endócrino. Avaliamos o perfil proliferativo das células presentes nas ilhotase ainda, a distribuição das células α e β por meio da marcação de glucagon e insulina no pâncreas de fetos de 19 dias. Analisamos por RT-qPCRa expressão dos fatores de transcrição *Pdx1* e *Pax4* que controlam o desenvolvimento e diferenciação das células β pancreáticas. O modelo utilizado foi o de gestação complicada por diabetes *mellitus*do tipo 1 (DM1), desenvolvido por nosso grupo, quimicamente induzido por aloxana sem tratamento de reposição insulínica, em camundongos. Observamos que a marcação de panlaminina e das cadeias α1 e γ1 das lamininas é mais fraca no pâncreas endócrino dos fetos de mães hiperglicêmicas, quando comparado ao grupo controle. Por outro lado, vimos um aumento na deposição da integrina α3 na membrana basal das ilhotas pancreáticas dos fetos gerados sob condições de hiperglicemia materna. O índice proliferativo das células endócrinas, observado por imuno-histoquímica para PCNA, também é menor nesse grupo. Observamos um aumento da área de ilhotas fetais imunomarcadas para a insulina, indicando aumento na massa de células β nessas ilhotas, enquanto que a área imunomarcada para glucagon estava com marcação menos intensa no grupo experimental comparado ao controle. Identificamos que aexpressão relativa de *Pdx1* é menor no pâncreas do grupo experimental comparado a expressão nos animais do grupo controle, enquanto a expressão de *Pax*4 está aumentada. Concluímos por meio de nossas abordagens histoquímicas que a hiperglicemia materna altera a morfogênese do pâncreas endócrino fetal modificando o

padrão de deposição de moléculas da membrana basal peri-ilhotas, promovendo uma diminuição da atividade proliferativa das células endócrinas, associada a alterações na expressão de fatores de crescimento importantes para o estado diferenciado e proliferativo das células β. Essas células apresentam aumento da massa funcional identificada pelo aumento da deposição de insulina no tecido pancreático.

PALAVRAS-CHAVE: Diabetes *Mellitus* tipo 1. Matriz Extracelular**.** Reprogramação Fetal. Histologia. Biologia do Desenvolvimento.

ABSTRACT

DIAS, C. Research about occurrence of fetal reprogramming in the development of endocrine pancreas in animal model of type 1 diabetes *mellitus* [dissertation (Master´s degree in System Biology)], Institute of Biomedical Sciences; University of São Paulo, São Paulo; 2019.

Previous studies from our lab and others have shown that adverse conditions during intrauterine development promotes molecular and structural changes in vital organs and systems which may alter on their function in the adult individuals. Hyperglycemia impacts on fetal development by modifying important biological processes, such as the pattern of synthesis and deposition of extracellular matrix (ECM) components. ECM cooperates in pancreatic branching and morphogenesis and little is known about the effect of maternal hyperglycemia on the pancreas' ECM during development. We investigate through immunohistochemistry, how severe maternal hyperglycemia modifies the distribution of panlaminin, lamininsα1 and γ1 chains and integrin α3, ECM molecules that play a key role in the differentiation of the endocrine pancreas. We evaluate the proliferative index of islet cells and, α and β cells distribution, by glucagon and insulin fetal (E19.0) pancreas staining. We analyzed by RT-qPCR the expression of the *Pdx1* and *Pax4* transcription factors that control the development and differentiation of pancreatic β cells. The model used was created by our group, a pregnancy model complicated by type 1diabetes *mellitus* (T1D) chemically induced by alloxan without treatment of insulin replacement, in mice. We observed that the labeling of panlaminin and laminins α1 and γ1 chains is weaker in the endocrine pancreas of the fetuses from hyperglycemic mothers. On the other hand, integrin α3 deposition increased in the basement membrane of the pancreatic islets of the fetuses generated under maternal hyperglycemia. Immunohistochemistry for PCNA showed lower proliferative index of endocrine cells. There was an increase in the area of immunolabeled fetal islets indicating an increase in β-cell mass in these islets; whereas the glucagon-immunolabeled area was smaller in the experimental group compared to the control group. The relative expression of *Pdx1* was lower in the pancreas from the experimental group, and the *Pax4* expression was increased. We conclude from our histochemical approaches that maternal hyperglycemia alters fetal endocrine pancreas morphogenesis by modifying the pattern of peri-islet basement membrane molecules, promoting a decrease in endocrine cell proliferative activity associated with changes in the expression of important growth factors for the differentiated and proliferative state of the β-cells. These cells have increased functional mass identified by increased insulin deposition in pancreatic tissue.

KEY WORDS: Type 1 Diabetes *Mellitus.* Extracellular Matrix. Fetal Reprogramming. Histology. Development Biology.

1 INTRODUÇÃO

O pâncreas é uma glândula mista essencial para o metabolismo de nutrientes. Possui uma porção exócrina, composta por acinos que secretam enzimas digestivas e por uma porção endócrina, formada pelas ilhotas pancreáticas que abrigam as células α, β, γ, ε e PP responsáveis pela homeostase da glicose (2). Em camundongos, o desenvolvimento pancreático torna-se morfologicamente evidente em torno do 9º dia embrionário, quando a superfície epitelial começa a se formar, alongando-se em ramos. A Matriz extracelular (MEC) participa diretamente do processo de morfogênese do pâncreas promovendo adesão das células precursoras pancreáticas, e pela interação de seus componentes com vias de sinalização importantes para a diferenciação e proliferação celular (2).

O Retardo de crescimento intrauterino é uma alteração frequente em modelos murinos de gestação complicada por diabetes. De modo geral, os fetos apresentam baixo peso corpóreo e menor volume pancreático, embora a porcentagem de tecido endócrino esteja aumentada, o que pode ser considerado um quadro de insuficiência pancreática (3).

Esses fenômenos adaptativos são considerados mecanismos de reprogramação fetal (4). A teoria da reprogramação do desenvolvimento embrionário e fetal foi postulada por David J. Baker no início dos anos 90 (4). Por meio de um estudo *coorte*, Baker mostrou que filhos de mães malnutridas no período perinatal, têm maior predisposição a desenvolver doenças cardiovasculares e metabólicas ao longo da vida adulta, devido a mecanismos adaptativos adotados durante a vida intrauterina para garantir sua sobrevivência (4). Barker também correlacionou o surgimento de hiperinsulinemia e resistência à insulina em indivíduos adultos ao diabetes materno, indicando que essa doença pode estimular a reprogramação nos fetos (4).

O Diabetes *mellitus* (DM) é caracterizado pela hiperglicemia ocasionada por defeitos na secreção e/ou ação da insulina nas células alvo (3). São classificados três tipos principais, sendo eles: Tipo 1 (DM1), Tipo 2 (DM2) e Diabetes Gestacional (DMG) (5)(6)(7).O desenvolvimento e a progressão dos três tipos de diabetes apresentam componentes genéticos, porém, fatores ambientais também estão intimamente relacionados à disfunção e/ou morte das células β pancreáticas produtoras de insulina (7). O DM1 tem baixa prevalência, mas aproximadamente 86.000 individuos desenvolvem essa doença a cada ano mundialmente (7). Em decorrência da hiperglicemia crônica podem ocorrer sérias complicações, dentre elas desordens reprodutivas e comprometimento da organogênese em fetos gerados por mães hiperglicêmicas (7)(8). A MEC participa diretamente da morfogênese, ramificação e do comportamento celular do pâncreas em desenvolvimento (9).As lamininas111, 211, 221, 411

e 421 presentes na MEC pancreática de roedores são essenciais para a adesão e proliferação, como também para a secreção de insulina pelas células β (10). Já a integrina α3 atua como receptor para lamininas e desempenha um papel essencial no desenvolvimento das células β regulando sua sobrevivência e função (11).

Dada a importância da MEC para o desenvolvimento e função adequados do pâncreas (12) e sabendo que o DM1 é uma condição que pode influenciar o desenvolvimento fetal alterando os componentes de MEC (12)(13)(14), hipotetizamos que a hiperglicemia materna poderia modificar a deposição das cadeias α1 e γ1 das principais lamininas presentes no pâncreas fetal, assim como da integrina α3 no pâncreas endócrino dos fetos, e ter impacto na expressão de fatores de transcrição importantes para a diferenciação e proliferação de células β pancreáticas fetais, como *Pdx1* e *Pax4*.

Nossa hipótese foi investigada em pâncreas de fetos de camundongos de 19 dias provindos de um modelo de gestação complicada por hiperglicemia, tipo DM1 de curto prazo (30-50 dias) quimicamente induzido sem reposição insulínica (13)(15)(16).

7 CONCLUSÃO

Concluímos que a hiperglicemia materna 30-50 dias promove alterações no desenvolvimento do pâncreas endócrino fetal de camundongos, modificando diferencialmente o padrão de deposição de moléculas da membrana basal das ilhotas, associado a diminuição da atividade proliferativa das células endócrinas com aumento da massa funcional de células β. Acreditamos que o conjunto dessas alterações seja um indício de reprogramação das células do pâncreas endócrino, que levam a modificações na morfologia do órgão e de seu funcionamento. O estudo aprofundado desses achados iniciais são necessários para comprovar a reprogramação por parte dessas células e os efeitos a longo prazo.

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