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Technical note

Gestational diabetes affects fetal autophagy

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ABSTRACT

Autophagy is a catabolic process involved in the preservation of energy homeostasis and its dysregulation has been implicated in the development of metabolic disorders, including diabetes mellitus. Gestational diabetes mellitus represents a risk for fetal morbidity and mortality. The present study focuses on the autophagy process in human diabetic placenta and fetal pancreas, compared with controls. Analysis of the autophagy markers LC3, Beclin-1 and p62 suggests an impairment of the autophagy process in diabetic placentas. Results indicate an association between gestational diabetes and autophagy, emphasizing the importance of unravelling the mechanisms regulating this relationship.

1. Introduction

Gestational diabetes mellitus (GDM), a type of diabetes that occurs during pregnancy, affects both mother and fetus [1], alters placenta and fetal pancreas [2–4] and represents a risk factor for fetal mortality and morbidity [5]. During pregnancy, glucose flows from mother to fetus across the placenta barrier through glucose transporters. Fetal glucose homeostasis is then regulated by fetal pancreatic beta-cell function. In the diabetic fetus, pancreas characteristics are related to alterations in the placental uptake of nutrients and to the high maternal and fetal glucose levels [6–8].

An intracellular process sensitive to glucose levels is autophagy, a self-degradative mechanism that has been related to the development of metabolic disorders [9]. During the autophagy process, senescent or damaged or redundant organelles or portion of cytoplasm are sequestered in a double-membrane vesicle called autophagosome that fuse with lysosomes to allow material breakdown and recycling [10].

Although previous studies suggested an association among placental autophagy, maternal metabolic status [11] and glucose concentration [12], no information is available on the expression of autophagy markers in human GDM. Previous non-obstetrical studies investigated the relationship between autophagy and diabetes and several reviews suggest a role of autophagy in the pathophysiology of diabetes mellitus [13–17]. Here we aim to evaluate the expression of autophagy markers in fetal pancreas and placenta from human GDM pregnancy.

2. Methods

2.1. Cases series

Stillborn cases from the autopsies archive were randomly selected (n = 7) from singleton full-term pregnancies which ended with a stillbirth with no other known gestational complication except gestational diabetes. Controls were age-matched randomly selected stillborn cases from pregnancies without GDM and classified as “unexplained stillborn”. For each stillborn case the fetal pancreas and placenta were evaluated.

For the liveborn cases the placentas from full-term singleton GDM pregnancies (n = 16) and uneventful pregnancies (n = 16) were prospectively collected.

Clinical characteristics are summarized in Supplementary Table 1.

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2.2. Autophagy evaluation

The autophagy markers Beclin-1, microtubule associated protein 1 light chain 3 (LC3) and p62 were evaluated.

Methods used were immunofluorescence, Western blot and quantitative real time RT-PCR, [18,19]. Methodological details are summarized in Supplementary file 1 and Table 2.

3. Results and discussion

3.1. GDM increases fetal pancreas and placenta expression of the key autophagy marker LC3

We initially ascertained if LC3 expression was altered in stillborn-GDM.

LC3 expression was observed in beta-cells of all fetal pancreases. When comparing the expression of LC3 in pancreas from pregnancies with and without GDM, we observed a significantly increased number of positive beta-cells in GDM (Fig. 1). This was related to the known hypertrophy of the Langerhans islets due to beta-cell hyperplasia (Fig. 1). Previous studies demonstrated that autophagy is crucial in the normal pancreas for the maintenance of beta-cells architecture and for insulin secretion and autophagy alteration is related to diabetes [20–22]. During pregnancy, it is established that maternal diabetes promotes fetal beta-cell formation [23] leading to beta-cell hyperplasia and fetal hyperinsulinemia [24]. It is also known that increased insulin resistance leads to autophagy up-regulation [14], probably to protect beta-cells against death and to allow compensatory cell expansion [14]. It is therefore conceivable that the increased fetal pancreas autophagy marker expression observed in GDM in this study represents an effect related to Langerhans morphology associated with fetal hyperglycemia and hyperinsulinemia (i.e. increased beta-cells number).

As fetal beta-cell hyperplasia in GMD pregnancy is known to be related to fetal and maternal hyperglycemia, which in turn is linked to placental alteration, we next evaluated the expression of LC3 in placentas. LC3 fluorescent dots appeared higher in syncytiotrophoblast from stillborn-GDM compared to non-GDM cases (Fig. 1), suggesting an increased autophagy in diabetic placenta.

3.2. Diabetes affects the expression of the autophagy markers in human placenta

We then assessed if the modification of LC3 expression in GDM placenta was caused by an increase in the autophagy process or if it was related to LC3 accumulation due to decreased vesicles degradation. We analyzed the expression of three different autophagy markers in liveborn cases.

Beclin-1 was significantly lower in GDM than in non-diabetic placentas ($p = 0.04$; Fig. 2). On the contrary, LC3-II was higher in GDM than in non-diabetic placentas both considering LC3-II in the ratio over beta-actin ($p = 0.01$; Fig. 2) and over LC3-I (data not shown). p62 was higher in GDM than in non-diabetic placentas

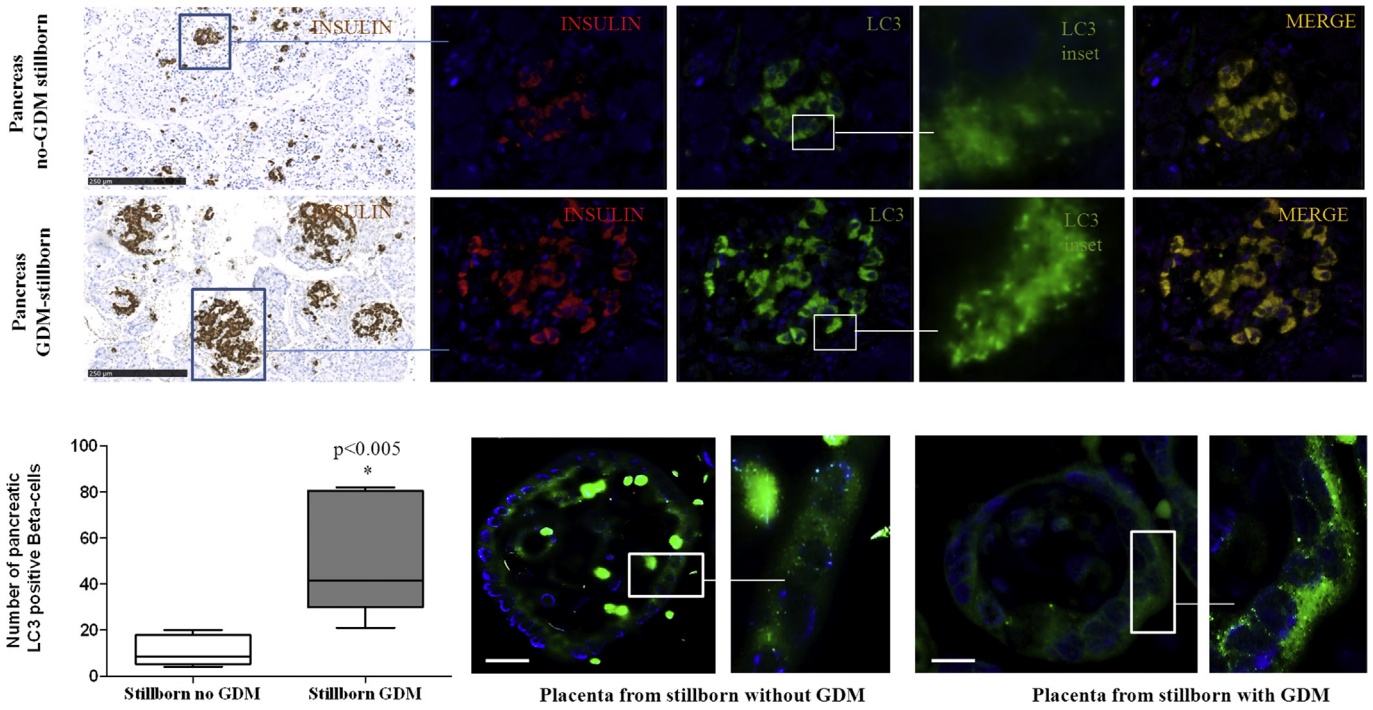

Fig. 1. LC3 expression is increased in stillborn GDM fetal pancreas and placenta.
Representative examples of autophagy localization in fetal pancreatic cells show that LC3 (in green) co-localizes with insulin antibody (in red) in beta-cells of Langerhans islets (merge in yellow), indicating that autophagy was present in fetal pancreatic Langerhans islets whose cells secrete insulin. Note the increased dimension of Langerhans islets in pancreas obtained from GDM pregnancy. In the insets higher magnification of LC3 expression is shown, emphasizing LC3 dots, suggesting the activation of the autophagy process in beta-cells. Scale bar = 250 μm.
The comparison between the number of LC3 positive cells in the biggest Langerhans islet in pancreas from GDM-stillborn ($n = 4$) and stillborn fetuses from pregnancies without GDM ($n = 4$) shows significantly increased number of LC3 positive beta-cells ($p < 0.005$). Data are presented as a boxplot: the median is horizontal lines in the box; the upper line of the box represents the upper quartile, the lower line of the box represents the lower quartile. Whiskers represent the maximum and minimum value.
Representative example of autophagy expression in placental villous syncytiotrophoblast (the placental site involved in the fetal-maternal exchange of nutrient) in stillborn from pregnancy affected by gestational diabetes and pregnancies without diabetes is shown in the lower panel. Scale bar = 15 μm.
The aspect of the fluorescence helps distinguishing the activation of the autophagy process: LC3 homogeneous and diffuse signal reflects latent activity (ubiquitous basal autophagy), whereas an active autophagy process is visualized as dots. Note in these cases the wide distribution of dots in GDM-stillborn compared with stillborn without GDM.