Mammalian target of rapamycin inhibition abrogates insulin-mediated mammary tumor progression in type 2 diabetes

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Abstract

Type 2 diabetes increases breast cancer risk and mortality, and hyperinsulinemia is a major mediator of this effect. The mammalian target of rapamycin (mTOR) is activated by insulin and is a key regulator of mammary tumor progression. Pharmacological mTOR inhibition suppresses tumor growth in numerous mammary tumor models in the non-diabetic setting. However, the role of the mTOR pathway in type 2 diabetes-induced tumor growth remains elusive. Herein, we investigated whether the mTOR pathway is implicated in insulin-induced mammary tumor progression in a transgenic mouse model of type 2 diabetes (MKR mice) and evaluated the impact of mTOR inhibition on the diabetic state. Mammary tumor progression was studied in the double transgenic MMTV-Polyoma Virus middle T antigen (PyVmT)/MKR mice and by orthotopic inoculation of PyVmT- and Neu/ErbB2-driven mammary tumor cells (Met-1 and MCNeuA cells respectively). mTOR inhibition by rapamycin markedly suppressed tumor growth in both wild-type and MKR mice. In diabetic animals, however, the promoting action of insulin on tumor growth was completely blunted by rapamycin, despite a worsening of the carbohydrate and lipid metabolism. Taken together, pharmacological mTOR blockade is sufficient to abrogate mammary tumor progression in the setting of hyperinsulinemia, and thus mTOR inhibitors may be an attractive therapeutic modality for breast cancer patients with type 2 diabetes. Careful monitoring of the metabolic state, however, is important as dose adaptations of glucose- and/or lipid-lowering therapy might be necessary.

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Introduction

Type 2 diabetes is a growing health problem affecting more than 20 million people in the United States. Recently, the disease has been linked to an increased breast cancer risk and mortality (Larsson et al. 2007, Xue & Michels 2007, Barone et al. 2008). While all three hallmark features of type 2 diabetes (hyperinsulinemia, hyperglycemia, and hyperlipidemia) might be involved in this effect (Lann & LeRoith 2008), we have shown that insulin is predominantly responsible for accelerated tumor development and growth in the setting of type 2 diabetes (Fierz et al. 2010, Novosyadlyy et al. 2010a). The promoting action of insulin on tumor growth is primarily mediated by the insulin receptor (IR) and/or the insulin-like growth factor 1 receptor (IGF1R). However, the intracellular signal transduction pathways implicated in this effect remain unidentified. Our previous study demonstrated that tumor tissue in diabetic mice has increased activity of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway (Novosyadlyy et al. 2010a), suggesting a role of this pathway in the accelerated tumor growth induced by hyperinsulinemia. The oncogenic activity of Akt may potentially result from the inactivation of numerous proapoptotic proteins (Bad, caspase-9, and GSK3b), cell cycle inhibitors (p21 and p27), products of tumor suppressor genes (FOX proteins, p53), and induction of signaling...
through nuclear factor \( \kappa B \) (NF-\( \kappa B \)) or the mammalian target of rapamycin (mTOR) pathway (Manning & Cantley 2007). In this study, we focused on the mTOR pathway due to the following reasons: a) its oncogenic role is well documented (Hynes & Boulay 2006, Guerlin & Sabatini 2007); b) mTOR inhibitors are already in clinical use as anti-tumor agents (Malizizza & Hsu 2008, Dancey et al. 2009, Yang et al. 2010); c) the role of the mTOR pathway in the regulation of carbohydrate and lipid homeostasis remains incompletely understood, and the metabolic consequences of pharmacological mTOR blockade in the setting of type 2 diabetes are largely unknown.

To study the effect of mTOR blockade on type 2 diabetes-induced mammary tumor progression, we employed a hyperinsulinemic mouse model of type 2 diabetes, the female MKR mice. These mice overexpress dominant negative IGF1Rs in the skeletal muscle, which inactivate the endogenous IRs and IGF1Rs (Fernandez et al. 2001). This leads to primary insulin resistance in the skeletal muscle as well as to secondary insulin resistance in fat and liver resulting in early-stage type 2 diabetes. The diabetic phenotype of the female MKR mice is characterized by severe hyperinsulinemia but only mild hyperglycemia and dyslipidemia (Novosyadlyy et al. 2010a). As hyperinsulinemia is the predominant metabolic abnormality in female MKR mice, these mice serve as an ideal model to study the effect of mTOR inhibition on insulin-mediated mammary tumor progression. To block the mTOR pathway, we used the potent mTOR inhibitor rapamycin, a macrolide isolated from Streptomyces hygroscopicus (Vézina et al. 1975, Heitman et al. 1991). This compound was approved by the FDA as an immunosuppressive drug to prevent rejection in patients after organ transplantation (Cowan & Heizer 2000) and has a potent anti-tumor activity (Guerlin & Sabatini 2007). To induce mammary tumors, we used two different approaches involving the Polyoma Virus middle T (PyVmT) and the Neu/ErbB2 oncogenes, both of which are stimulated in a hyperinsulinemic state (Novosyadlyy et al. 2010a) and have been shown to be responsive to rapamycin treatment (Liu et al. 2005, Namba et al. 2006, Mosley et al. 2007). We found that chronic treatment with rapamycin was able to fully abrogate insulin-mediated mammary tumor progression in a type 2 diabetic milieu, despite a worsening of the carbohydrate and lipid metabolism. This suggests that the mTOR arm of the PI3K/Akt pathway is a key mediator of accelerated mammary tumor growth induced by hyperinsulinemia. Thus, in patients with type 2 diabetes and breast cancer, mTOR inhibition is a promising treatment option; however, a possible worsening of the metabolic state might lead to an increased requirement of glucose- and/or lipid-lowering treatments.

Materials and methods

Animals

All mice used in the study were on the FVB/N background. The MKR\(^{+/+}\) (MCK-KR-hIGF-IR) mice and mouse mammary tumour virus (MMTV)-PyVmT\(^{+/−}\) mice have been generated and characterized previously (Guy et al. 1992, Fernandez et al. 2001). The mice were kept on a 12 h light:12 h darkness cycle and had access to a standard mouse chow and fresh water ad libitum. The Mount Sinai School of Medicine AAALAC-accredited animal facility provided animal care and maintenance.

Transgenic tumor model

PyVmT\(^{+/−}\) male mice were crossed with either MKR\(^{+/+}\) or wild-type (WT) female mice to generate PyVmT\(^{+/−}\) or PyVmT\(^{+/−}/MKR^{+/+}\) female mice, respectively. Rapamycin (LC Laboratories, Woburn, MA, USA) in PBS containing 4% ethanol, 5% Tween 80 (Fisher Scientific, Pittsburgh, PA, USA), and 5% polyethylene glycol (PEG) 400 (Fisher Scientific) was administered i.p. at a dose of 0.5 mg/kg body weight per day from 4 to 6 weeks of age. Control mice received an equal amount of vehicle (PBS containing 4% ethanol, 5% Tween 80, and 5% PEG 400). Before killing at 6 weeks of age, blood was collected for metabolic assays (see below). Both inguinal #4 mammary glands were carefully excised. One mammary gland was subjected to whole mount analysis (see below), the other gland was snap frozen in liquid nitrogen and stored at −80°C for further molecular analyses.

Syngeneic orthotopic tumor models

Met-1 mammary tumor cells were derived from MMTV-PyVmT (FVB/N) transgenic mice (Borowsky et al. 2005), and MCNeuA mammary tumor cells were derived from MMTV-Neu (FVB/N) transgenic mice (Campbell et al. 2002). Cell preparation and injection were performed as described previously (Fierz et al. 2010, Novosyadlyy et al. 2010a). Two weeks after cell inoculation, rapamycin (0.5 mg/kg body weight per day i.p.) or vehicle treatment was initiated and continued for 14 days. To monitor tumor growth, mammary fat pads were examined by finger palpation. The tumor volume was measured in a three-coordinate system using calipers and calculated by the formula: \(\frac{4}{3}\pi r_1 r_2 r_3\) (\(r = \text{radius}\)).
Body weight and food intake were determined twice a week. Blood glucose levels were measured once a week with an automated glucometer (Elite, Bayer). Plasma insulin and serum triglyceride levels were measured by ELISA (Mercodia AB, Upsala, Sweden) and a triglyceride quantification kit (BioVision Inc., Mountain View, CA, USA) respectively. A glucose tolerance test was performed after 6 h of fasting. A glucose bolus of 1 g/kg body weight was administered i.p., and blood glucose was measured from the tail vein immediately before and 15, 30, 60, and 120 min after glucose injection. Body composition was measured using an EchoMRI 3-in-1 NMR system (Echo Medical Systems, Houston, TX, USA).

Protein extraction and western blot analysis
Protein extraction and western blot analysis were performed as described previously (Fierz et al. 2010, Novosyadlyy et al. 2010a). Phospho(Ser235/236) and total S6 ribosomal protein (S6rp), phospho(Ser473) and total Akt, phospho-IR substrate (IRS)-1 (Ser636/639), phospho-IGF1Rβ(Tyr1135/36), 4E-BP1, and proliferating cell nuclear antigen (PCNA) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). IR and cyclin D1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The IRS-1 antibody was obtained from Millipore (Billerica, MA, USA). The β-actin antibody was from Sigma. Densitometric analysis was performed using MacBAS V2.52 software (Fuji PhotoFilm, Inc., Valhalla, NY, USA).

Apoptosis assay
Met-1 orthograft tumors were carefully excised, fixed in 4% paraformaldehyde overnight, and embedded in paraffin. Tumor sections were processed with the ApopTag In situ Apoptosis Detection kit (Chemicon, Temecula, CA, USA) according to the manufacturer’s instructions. Images were taken using the Olympus AX70 microscope coupled to the DP71 digital camera (Olympus, Central Valley, PA, USA) in combination with the computer software DP Manager version 3.1.1.208 (Olympus). The amount of apoptotic cells was determined by counting the number of terminal dUTP nick end labelling (TUNEL)-positive cells in ten fields (400×) of each tumor section.

Whole mount analysis of mammary gland
Whole mount analysis of the mammary gland was performed as described previously (Novosyadlyy et al. 2010a). In brief, mammary glands were fixed on a glass slide, hydrated, stained in carmine alum, dehydrated, cleared in xylene (Fisher Scientific), and coated with Mount Quick Mounting Medium (Daido Sangyo Co., Tokyo, Japan). Quantification of the hyperplastic mammary lesions was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analyses
Results are expressed as mean±S.E.M. Statistical analyses were conducted using a Student’s t-test. When more than two values were compared, ANOVA was applied followed by a Fisher’s test. A P value <0.05 was considered significant.

Results
Rapamycin exacerbates the metabolic state in WT and MKR mice
Female MKR mice develop a mild form of type 2 diabetes with severe insulin resistance and hyper-insulinemia but only moderate dysglycemia and dyslipidemia (Novosyadlyy et al. 2010a). To study the effect of chronic rapamycin treatment on diabetic (MKR) and non-diabetic WT mice, we administered rapamycin at a dose of 0.5 mg/kg body weight per day i.p. This dose has been shown to be effective in inhibiting mammary tumor progression and achieves similar blood levels as recommended for clinical use in humans (Meier-Kriesche & Kaplan 2000, Phung et al. 2007).

MKR mice display reduced body weight and body adiposity than WT mice (Novosyadlyy et al. 2010a). Chronic treatment with rapamycin had no effect on body weight in adult mice (10–13 weeks of age; Fig. 1A) and mildly inhibited body weight gain during the growth phase (4–6 weeks of age) (data not shown). Whole body fat was increased after 2 weeks of treatment in both WT and MKR mice (Fig. 1B), whereas food intake was unchanged by the treatment (data not shown). Moreover, rapamycin treatment led to a further increase in glucose levels in MKR mice, while no effect was observed in WT mice (Fig. 1C). When challenged with an exogenous glucose bolus, both WT and MKR mice showed a marked worsening of their glucose tolerance (Fig. 1D). Consistent with previous findings in normoglycemic P. obesus (Fraenkel et al. 2008), chronic rapamycin treatment led
PyVmT-induced mammary tumors are known to be responsive to pharmacological mTOR inhibition (Namba et al. 2006). We have shown previously that hyperinsulinemia mediates an acceleration of hyperplasia formation in the double transgenic PyVmT/MKR mice at 6 weeks of age (Novosyadlyy et al. 2010a). In this study, we aimed to investigate whether inhibiting mTOR by rapamycin would be sufficient to block the accelerated formation of hyperplastic mammary lesions. As shown in Fig. 2A and B, PyVmT/MKR mice displayed an accelerated development of hyperplastic mammary lesions than non-diabetic PyVmT mice. When chronically treated with rapamycin (0.5 mg/kg body weight per day i.p.), starting 2 weeks prior to killing, the development of mammary lesions was suppressed to similar levels in PyVmT/MKR and PyVmT mice, suggesting that mTOR inhibition is able to fully abrogate insulin-mediated hyperplasia formation (Fig. 2A and B). It was observed that the absolute reduction in the size of the hyperplastic lesions was more pronounced in

**Rapamycin treatment abrogates the accelerated formation of hyperplastic mammary lesions in PyVmT/MKR mice**

To study the effect of rapamycin on mammary tumor development, a transgenic approach was chosen, whereby MKR mice were interbred with MMTV-PyVmT transgenic mice (Guy et al. 1992). The PyVmT model of mammary tumor development shares many morphological and biochemical similarities with human breast cancer (Lin et al. 2003), and.
PyVmT/MKR mice than that in PyVmT mice (Fig. 2C). The hyperinsulinemic phenotype in PyVmT/MKR mice led to an up-regulation of the mTOR pathway in hyperplastic mammary tissue as shown by an increase in phosphorylation of S6rp, a downstream effector of mTOR and S6 kinase (Fig. 3A and B). However, despite the further activation of the mTOR pathway in PyVmT/MKR mice, treatment with rapamycin was able to block S6rp phosphorylation to the same extent in PyVmT/MKR mice as it did in PyVmT mice (Fig. 3C and D). To study the effect of rapamycin on the 4E-BP1/eIF4E arm of the mTOR pathway, the differentially phosphorylated 4E-BP1 isoforms were resolved by migration in SDS-PAGE. The extent of phosphorylation of 4E-BP1 was quantified as a ratio of the more phosphorylated and thus more slowly migrating isoforms over total 4E-BP1. As shown in Fig. 3C and D, treatment with rapamycin led to a mild decrease of 4E-BP1 phosphorylation to the same extent in hyperplastic mammary tissue from PyVmT mice as well as from PyVmT/MKR mice.

Studies have shown that a negative feedback loop exists from the mTOR–S6 kinase pathway to the upstream, insulin-responsive IRS–PI3K–Akt pathway (Harritongton et al. 2004, Um et al. 2004). Thus, inhibition of mTOR may lead to an increased activation of the IRS/PI3K/Akt axis, and this might compromise the effectiveness of mTOR inhibition on tumor progression in a type 2 diabetic state. We therefore evaluated the activation of the IR/IGF1R/Akt signaling pathway in hyperplastic mammary tissue. Consistent with our previous findings, the IR/IGF1R/Akt pathway was more activated in hyperplastic mammary tissue derived from PyVmT/MKR mice than that from PyVMT mice (Fig. 3C and D). In line with an abrogation of the negative feedback loop of S6 kinase on IRS-1, the inhibitory phosphorylation of IRS-1 on Ser636/639 tended to be reduced by rapamycin treatment in PyVmT/MKR mice (P = 0.066, ANOVA), whereas IR/IGF1R activation was not affected by the treatment. However, despite the tendency toward a reduced inhibitory phosphorylation of IRS-1, Akt activation was not significantly changed by the treatment (Fig. 3C and D).

In summary, pharmacological blockade of the mTOR pathway was able to fully abrogate insulin-mediated acceleration of hyperplasia formation in PyVmT/MKR mice mainly by inhibiting the S6 kinase/S6rp branch of the mTOR pathway. Furthermore, rapamycin treatment did not lead to a further activation of the PI3K/Akt signaling pathway in hyperplastic mammary tissue derived from PyVmT/MKR mice.

Rapamycin treatment attenuates insulin-mediated mammary tumor growth of syngeneic Met-1 and MCM10A orthografts

To assess the impact of rapamycin on the growth of fully transformed tumor cells, we used two syngeneic
Figure 4 Pharmacological mTOR inhibition abrogates insulin-mediated tumor growth of Met-1 and MCNeuA orthografts. (A and B) At 8 weeks of age, 0.5×10^6 Met-1 cells or 10^6 MCNeuA cells were inoculated into the #4 mammary fat pad. Two weeks after cell injection, WT and MKR mice were treated with rapamycin (Rapa) (0.5 mg/kg body weight/day i.p.) or vehicle (V) for 14 days. Tumor size was measured twice per week using calipers. Arrow (Rapa) (0.5 mg/kg body weight per day i.p.) for 14 days. Both Met-1- and MCNeuA-induced tumor growth was accelerated in MKR mice compared to WT mice (Fig. 4A and B). In WT mice, mTOR inhibition led to a significant reduction in tumor size; in MKR mice, however, insulin-mediated tumor progression was fully abrogated in both the tumor models (Fig. 4A and B). It was observed that the absolute tumor reduction was more pronounced in MKR mice than in WT mice (Fig. 4C and D). Similar to our observation in

mouse mammary tumor cell lines, Met-1 and MCNeuA, which are derived from MMTV-PyVmT (FVB/N) and MMTV-Neu (FVB/N) transgenic mice respectively (Campbell et al. 2002, Borowsky et al. 2005). This allowed us to study the effect of rapamycin treatment on insulin-mediated tumor progression induced by two different approaches involving the PyVmT and Neu/ErbB2 oncogenes, both of which are known to be responsive to rapamycin (Liu et al. 2005, Namba et al. 2006, Mosley et al. 2007). At 8 weeks of age, 0.5×10^6 Met-1 cells or 10^6 MCNeuA cells were injected orthotopically into the #4 mammary fat pad of adult female mice. Two weeks after cell inoculation, rapamycin treatment was initiated (0.5 mg/kg body weight per day i.p.) for 14 days. Both Met-1- and MCNeuA-induced tumor growth was accelerated in MKR mice compared to WT mice (Fig. 4A and B). In WT mice, mTOR inhibition led to a significant reduction in tumor size; in MKR mice, however, insulin-mediated tumor progression was fully abrogated in both the tumor models (Fig. 4A and B). It was observed that the absolute tumor reduction was more pronounced in MKR mice than in WT mice (Fig. 4C and D). Similar to our observation in

hyperplastic mammary tissue of PyVmT/MKR mice, hyperinsulinemia led to an increased activation of the mTOR/S6 kinase pathway in tumor tissue as demonstrated by an increased S6rp phosphorylation (Fig. 5A--D). Moreover, treatment with rapamycin reduced S6rp phosphorylation to a similar extent in tumors derived from MKR and WT mice. Thus, the treatment was able to fully block the insulin-mediated activation of the mTOR pathway (Fig. 5A--D). Rapamycin treatment reduced 4E-BP1 phosphorylation to an equal or even greater extent in tumors derived from MKR mice than that from WT mice. However, in line with our findings in the transgenic model, the effect on 4E-BP1 activation was less pronounced than that on S6rp phosphorylation (Fig. 5A--D).

To determine whether the observed abrogation of tumor growth by rapamycin treatment was due to inhibition of cell proliferation, the levels of the

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**Figure 5** The increased activation of the mTOR pathway in Met-1 and MCNeuA tumor tissue derived from MKR mice is abrogated by rapamycin treatment. Proteins extracted from Met-1 (A) and MCNeuA (B) tumor tissue after treatment with rapamycin (Rapa) or vehicle (V) for 14 days were size fractioned by SDS-PAGE and immunoblotted with anti-phospho-S6rp (Ser235/236) and anti-4E-BP1 antibodies. Total level of proteins was determined by immunoblotting with antibodies directed against the respective total protein and β-actin. Hypo, hypophosphorylated isoform, hyper, hyperphosphorylated isoform of 4E-BP1. Each line represents a different animal. (C and D) The results of the densitometric analysis are presented as a fold increase compared to WT, vehicle-treated mice. Results are expressed as mean ± S.E.M. *P<0.05 between treated and untreated group, †P<0.05 between WT and MKR mice within the same treatment group (ANOVA).
proliferation marker PCNA were evaluated in Met-1 tumors. As expected, PCNA protein levels were significantly decreased by the treatment in Met-1 tumors grown in MKR and WT mice (Fig. 6A and B). To further evaluate whether rapamycin treatment abrogates tumor growth in MKR mice by induction of apoptosis, sections of Met-1 orthograft tumors were subjected to a TUNEL-based assay. The number of apoptotic cells was increased in tumors derived from MKR mice treated with rapamycin (Fig. 6C and D), whereas no difference could be observed in WT mice.

Taken together, pharmacological blockade of the mTOR pathway was able to abrogate insulin-mediated tumor growth of fully transformed tumor cells in the setting of type 2 diabetes, and tumor growth inhibition was induced by decreased cell proliferation and increased apoptosis.

**Discussion**

We demonstrated recently that type 2 diabetes in general and hyperinsulinemia in particular enhance the growth and development of mammary tumors independent of obesity (Fierz et al. 2010, Novosyadlyy et al. 2010a). Furthermore, our results indicate that this tumor-promoting activity is mediated by the activation of the IR and the IGF1R and further signal transduction through the PI3K/Akt pathway. mTOR, which is often deregulated in cancer (Guertin & Sabatini 2007), is one of the downstream signaling intermediates activated by Akt (Manning & Cantley 2007). Therefore, we rationalized that the effect of hyperinsulinemia on tumor growth may be attributed to an enhanced signaling through the mTOR pathway. Indeed, our data demonstrate that hyperplastic and tumor tissue extracted from type 2 diabetic mice exhibit an increased activity of the mTOR pathway. We further studied the effect of pharmacological mTOR inhibition on mammary tumor development and growth in the setting of type 2 diabetes. In accordance with previous reports (Liu et al. 2005, Namba et al. 2006, Mosley et al. 2007), we observed a significant reduction in mammary tumor progression in non-diabetic (WT) mice. Interestingly enough, the accelerated formation of precancerous hyperplastic lesions and tumor growth were completely abolished in rapamycin-treated diabetic (MKR) mice. It is important to note that the magnitude of tumor suppression was significantly higher in MKR mice than in WT mice treated with rapamycin. These data indicate that hyperinsulinemia fails to stimulate tumor growth when mTOR activity is blocked. These results link mTOR
and hyperinsulinemia mechanistically and provide a preclinical rationale for targeting mTOR in breast cancer, particularly in the setting of type 2 diabetes.

The mTOR/S6 kinase pathway has been shown to exert a negative feedback on the insulin signaling pathway via inhibition of IRS-1 (Harrington et al. 2004, Um et al. 2004). Thus, pharmacological inhibition of mTOR may lead to an increased activation of the PI3K/Akt axis in mammary tumors resulting in a limitation of the anti-tumor effectiveness of rapamycin treatment (O’Reilly et al. 2006). In this study, however, no further increase of the Akt pathway could be observed in hyperplastic mammary tissue derived from rapamycin-treated MKR mice. The reason for this observation could be that chronic rapamycin treatment inhibits, at least in part, the mTORC2 complex, which is responsible for the phosphorylation of Akt at Ser473 (Sarbassov et al. 2005, 2006). Thus, in type 2 diabetic MKR mice, chronic rapamycin treatment does not further activate the PI3K/Akt signaling axis in hyperplastic mammary tissue and is therefore fully effective as an anti-tumor agent.

Rapamycin treatment has been shown previously to reduce cell proliferation by inhibition of the cell cycle as well as by induction of apoptosis in mammary tumor tissue (Liu et al. 2005, Namba et al. 2006, Mosley et al. 2007). In this study, no significant effect on apoptosis as well as on cyclin D1 levels could be observed in non-diabetic mice, which may be due to the low-dose regimen used. Interestingly, in MKR mice, mTOR inhibition led to an increase in tumor cell apoptosis as well as to a decrease in cyclin D1 levels in mammary tumors. This suggests that in MKR mice, tumor tissue is more susceptible to the anti-tumor effects of rapamycin, and thus mammary tumors in type 2 diabetic individuals may be particularly responsive to pharmacological mTOR inhibition.

The impact of mTOR blockade on diabetes-related metabolic abnormalities is poorly understood. The mTOR pathway plays a critical role in adipogenesis (Wullschleger et al. 2006), and treatment with rapamycin has been shown to protect from high-fat diet-induced obesity (Chang et al. 2009a,b). Moreover, S6 kinase-deficient mice display reduced body adiposity (Um et al. 2004). Rapamycin has also been demonstrated to improve insulin signaling in vitro (Li et al. 1999). These data suggest that mTOR inhibition might have potential anti-obesity and/or insulin-sensitizing effects. However, several experimental and clinical studies indicate that pharmacological mTOR blockade may aggravate the metabolic disturbances in type 2 diabetes (Fraenkel et al. 2008, Chang et al. 2009b, Veilleux et al. 2010). Indeed, in obese animals with type 2 diabetes, chronic treatment with rapamycin worsened glucose tolerance and had variable effects on insulin levels (Fraenkel et al. 2008, Chang et al. 2009b). In humans, pharmacological mTOR inhibition led to hypertriglyceridemia, hyperglycemia, glucose intolerance, insulin resistance, and impairment of pancreatic β-cell response (Morrisset et al. 2003, Teutonico et al. 2005, Bellmunt et al. 2008, Johnston et al. 2008, Malizzi & Hsu 2008). In accordance with these reports, our data demonstrate that treatment with rapamycin resulted in an elevation of glucose and triglycerides in the circulation and significantly worsened glucose tolerance in MKR mice. However, the markedly elevated plasma insulin levels in MKR mice did not change significantly upon rapamycin treatment. It has been demonstrated that the mTOR pathway plays a pivotal role in maintaining β-cell mass and insulin secretion (Pende et al. 2000, Zahr et al. 2007, Rachdi et al. 2008). Therefore, it is plausible that in rapamycin-treated MKR mice, unaltered plasma insulin levels in the face of elevated blood glucose levels reflect initial stages of β-cell decompensation.

Intriguingly, treatment with rapamycin resulted in a significantly increased body adiposity in both WT and MKR mice. The mechanism mediating this effect is not understood but may potentially be attributed to a suppression of the mTOR/S6 kinase pathway in hypothalamic neurons. Indeed, it has been demonstrated previously that an increased S6 kinase activity in the mediobasal hypothalamus improves energy homeostasis and protects against diet-induced obesity and insulin resistance (Blouet et al. 2008).

Our results demonstrate that rapamycin exacerbates the metabolic derangements in type 2 diabetes as evident by impaired glucose and lipid homeostasis and altered body composition, all of which may have potential tumor-promoting activity (Vona-Davis et al. 2007, Lann & LeRoith 2008, Novosyadlyy et al. 2010b). However, despite a worsening of the diabetic state, these animals fail to demonstrate an accelerated tumor growth as observed in the vehicle-treated MKR mice. These data thus strongly suggest that hyperinsulinemia-mediated mTOR activation is the key pathophysiological mechanism of mammary tumor progression in type 2 diabetes. Therefore, targeting mTOR in breast cancer patients with type 2 diabetes might be a promising treatment strategy. A careful monitoring of the metabolic state, however, will be important, and a more aggressive anti-diabetic and/or lipid-lowering therapy might be necessary in this cohort of patients.
Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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