COMMENTARY

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Should diabetic women with breast cancer have their own intervention studies?

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Abstract

This commentary on 'Calorie restriction and rapamycin inhibit MMTV-Wnt-1 mammary tumor growth in a mouse model of postmenopausal obesity' by Nogueira *et al.*, published in this issue of *Endocrine-Related Cancer*, addresses the challenges of translating diet, exercise, and pharmacologic trials in diabetic mouse mammary tumor models to human studies. We propose that trials specifically designed to test such interventions in diabetic women with breast cancer would be valuable and informative.

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While the incidence of breast cancer in the United States is stable and overall mortality is declining (Berry *et al*. 2005), an epidemic of obesity-related type 2 diabetes (Ford *et al*. 2005) is expected to influence breast cancer outcomes in women with newly diagnosed breast cancer and also in the growing population of breast cancer survivors (Goodwin *et al*. 2002, Yeh *et al*. 2011). Obesity and diabetes have been linked to increased breast cancer risk and poor breast cancer outcomes (Coughlin *et al*. 2004, Xue & Michels 2007, Barone *et al*. 2008, Lipscombe *et al*. 2008); and animal models are needed to inform the development of trials testing dietary, exercise, and pharmacologic interventions designed to increase recurrence-free survival. Specifically, poor breast cancer outcomes have been linked to hyperinsulinemia (Goodwin *et al*. 2002). Realistic and tolerable interventions are needed that could mitigate the hyperinsulinemic state and slow breast cancer progression or increase latency. Although exercise has been demonstrated to reduce the hyperinsulinemic state in obese women who are not known to be diabetic (Ligibel *et al*. 2008), similar interventions have not been carried out in obese women known to be diabetic. Therefore, there is an unmet need for preclinical models to inform the development of clinical interventions. Specifically, do dietary, exercise, or pharmacologic interventions have an effect on breast cancer recurrence-free survival in diabetic patients?

Nogueira *et al*., published in this issue of *Endocrine-Related Cancer*, investigate the effects of calorie restriction, exercise, and the MTOR inhibitor rapamycin on mammary tumor latency and progression of syngeneic Wnt-1-driven mammary tumors in ovariectomized C57BL/6 mice made obese by 8 weeks of exposure to a high-fat diet (Nogueira *et al*. 2012). The preclinical model is based on the hypothesis that preexisting obesity in postmenopausal women promotes the progression of early breast cancer, in part, through MTOR signaling (Goodwin & Stambolic 2011). They tested if interventions in diet and/or exercise and MTOR interception would block breast cancer progression. The mice in this study exhibit elevated insulin and fasting glucose levels, suggesting that they are diabetic in addition to being obese and ovariectomized. Indeed, the C57BL/6 line used in this study is highly susceptible to type 2 diabetes, conjectured to be through absence of nicotinamide nucleotide transhydrogenase (Aston-Mourney *et al*. 2007). Prior models of insulin-related mammary carcinoma progression have been performed in nonobese hyperinsulinemic $MKR^{+/+}$ mice, which express two copies of a dominant-negative insulin-like growth factor 1 receptor (IGF1R) in muscle (Fernandez *et al*. 2001), resulting in a hyperinsulinemic state similar to early type 2 diabetes (Novosyadlyy *et al*. 2010). While these mouse models are similar with respect to hyperinsulinemia, the obese ovariectomized

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mice used in the Nogueira study (Nogueira *et al*. 2012) appear to be more closely matched to patients with obesity-induced diabetes. The new study therefore exhibits some similarity to the $MKR^{+/+}$ studies of LeRoith *et al*. (Fierz *et al*. 2010*a*,*b*), but with a twist, namely that the mechanisms of diabetes etiology differ in detail between the models and diet and exercise are studied in addition to pharmacological interventions. But do the obese diabetic mice give us the answers we expect and are they the answers we want to hear?

Nogueira *et al*., asked whether in obese, ovariectomized mice intervention with calorie restriction, exercise, or the MTOR inhibitor rapamycin affects time to palpable tumor and tumor weight at the endpoint (Nogueira *et al*. 2012). They also asked whether constitutively active MTOR ablates the beneficial effects of calorie restriction. The main conclusions are that calorie restriction or rapamycin, but not exercise, significantly reduced tumor growth relative to control mice. Furthermore, constitutive activation of MTOR ablated the inhibitory effects of calorie restriction on Wnt-1 mammary tumor growth. The authors conclude that MTOR inhibition may be a pharmacologic strategy to mimic the anticancer effects of calorie restriction and break the obesity – breast cancer progression link. Consistent with these conclusions, MTOR inhibition by rapamycin in $MKR^{+/+}$ mice also abrogates mammary tumor progression (Fierz *et al*. 2010*b*). The results obtained with obese ovariectomized mice could have significant implications on how future clinical intervention studies would be designed, because they support the hypothesis that exercise may not impact breast cancer risk in obese women, while the more difficult interventions of calorie restriction or MTOR inhibition may be better.

The study by Nogueira *et al*., suggests that calorie restriction increases latency time for breast cancer progression (Nogueira *et al*. 2012). In a human parallel, dietary modification in the Women's Intervention Nutrition Study tested the hypothesis that dietary fat reduction would increase relapse-free survival in women with early breast cancer undergoing standard cancer therapy (Chlebowski *et al*. 2006). In this study, there was a significant improvement of relapse-free survival associated with the lower fat diet (Chlebowski *et al*. 2006). Improved survival following breast cancer was also observed prospectively in physically active women with high vegetable–fruit intake regardless of obesity (Pierce *et al*. 2007), suggesting that a number of dietary intervention approaches can be taken. A primary analysis of the Women's Health Initiative calorie restriction study in breast cancer patients exhibited a trend toward improved relapse-free survival associated with a low-fat diet (Chlebowski *et al*. 2006). To be most informative, dietary interventions should follow the same parameters as exercise studies and monitor serum insulin levels (Ligibel *et al*. 2008), because breast cancer outcomes have been linked with this biomarker (Goodwin *et al*. 2002). Use of the insulin level as a biomarker may be one way forward to compare diet and exercise interventions.

MTOR inhibition appears to be a promising strategy in the Nogueira study (Nogueira *et al*. 2012). Nonetheless, it is important to make a distinction between MTOR inhibition and rapamycin. Rapamycin, an MTOR inhibitor also known as sirolimus, exhibits many well-known toxicities and is not recommended for prevention of breast cancer recurrence for many reasons, not the least of which is that it is immunosuppressive, in part through inhibition of lymphokine activity on T and B cells (Dumont *et al*. 1990, Kay *et al*. 1991). The mechanism of immunosuppression by rapamycin is in part due to interaction with immunophilin FKBP12, leading to immunosuppression (Wiederrecht *et al*. 1995). The potential risks of rapamycin related to immunosuppression in humans include risk of lymphoma and opportunistic infections (Ponticelli *et al*. 2001, Molinari *et al*. 2010). Rapamycin is also hyperlipidemic (Brattstrom *et al*. 1998, Ponticelli *et al*. 2001, Molinari *et al*. 2010). The safety bar is set higher for cancer prevention compared with cancer therapeutics and rapamycin-like drugs do not get past the bar. While the potential benefits of MTOR inhibition include possible reduction in cancer incidence (Sharp & Richardson 2011) and therefore could potentially play a role in improving relapse-free survival in breast cancer, rapamycin may not be the best approach. When considering a prevention drug, the bar is set high for allowable toxicity and rapamycin and related drugs lack a suitable safety profile.

If calorie restriction and MTOR inhibition worked, why did not exercise? Before we abandon exercise as an intervention, it is important to look at the insulin levels in the exercising mice. What was remarkable is that treadmill exercise in obese mice did not reduce insulin levels but actually increased them, in contrast to studies of obese breast cancer survivors (Ligibel *et al*. 2008). Consistent with this lack of insulin reduction, there was also no effect on tumor progression or latency. Is the treadmill lacking as a mouse model for human exercise?

If extrapolation is made from the Nogueira model to breast cancer, the hypothesis to be tested is that exercise

would have no impact on recurrence-free survival in obese breast cancer survivors (Nogueira *et al*. 2012). On the contrary, there are a number of studies in which physical activity has been associated with improved breast cancer prognosis, perhaps independent of obesity (Holmes *et al*. 2005, Abrahamson *et al*. 2006, Pierce *et al*. 2007). There has been at least one well-performed study in which exercise resulted in a reduction of insulin levels in nondiabetic overweight breast cancer survivors (body mass index (BMI) $\sim$30 kg/m^2; Ligibel *et al*. 2008). In contrast, exercise only marginally reduced insulin levels in a different study of healthy women aged 30–50, who were mostly premenopausal and exhibited lower BMI ($\sim$26 kg/m^2; Schmitz *et al*. 2002), suggesting greater benefits of exercise in obese postmenopausal women. In the obese postmenopausal breast cancer survivors exercise decreased not only insulin levels but also hip circumference (Ligibel *et al*. 2008). In the postmenopausal patients and in obese mice, exercise changed body fat distribution, but had no effect on body weight or body fat percentage. But contrary to the human study, insulin levels increased in obese exercising mice. Furthermore, serum leptin levels failed to fall in the exercising mice and serum adiponectin levels failed to increase, which suggest little impact of treadmill exercise intervention on hormonal pathways important for energy regulation in mice.

One possible explanation is that the treadmill did not work because of what appeared to be increased energy intake in the exercising mice. It is also very possible that the observed exercise results are related to the specific mechanisms of diabetes found in the C57BL/6 mouse strain and that the same effects may not be observed with other mouse models of diabetes. Therefore, it may be helpful to test the effects of exercise on MKR$^{+/+}$ mice. While it would be optimal to have human data to compare with the mouse studies, we currently lack exercise intervention studies in postmenopausal breast cancer patients with diabetes. It would be helpful to know the magnitude of the effect of exercise in diabetic subjects with breast cancer, as has been measured in glucose intolerant or obese subjects (Arciero *et al*. 1999, Sari *et al*. 2007). Regardless of the lack of effect of exercise in the obese ovariectomized mouse model, trials testing the impact of exercise in obese diabetic and nondiabetic women should be continued. The reduction of insulin levels in one intervention trial is promising (Ligibel *et al*. 2008). Furthermore, the type of exercise in the animal model and interaction with diet may matter.

In the ovariectomized mouse and the MKR$^{+/+}$ models MTOR inhibition by rapamycin abrogates the effects of insulin on mammary tumor progression at the expense of raising glucose and lipid levels (Fierz *et al*. 2010*b*). An alternative approach is hinted at by the success of insulin-sensitizing therapy with CL-316243, a specific β3-adrenergic receptor agonist attenuating type 2 diabetes-mediated mammary tumor progression in the MKR$^{+/+}$ model (Fierz *et al*. 2010*a*). It has been proposed that the biguanide drug metformin, which activates AMP kinase, could effectively reduce insulin levels in diabetic and nondiabetic subjects (Goodwin 2008). Because metformin induces AMP kinase, it functions indirectly as an MTOR inhibitor. Metformin reduces blood glucose and may be used in prediabetic and diabetic women with breast cancer in trials to prevent recurrence. Recently, metformin was demonstrated to safely reduce insulin levels of nondiabetic early breast cancer patients (Goodwin *et al*. 2008) and further intervention trials are ongoing in the MA.32 trial of metformin in women with early-stage breast cancer. Although metformin may lack the mechanistic specificity of MTOR inhibitors, it may help to circle back to study metformin in ovariectomized obese and MKR$^{+/+}$ mice to inform clinical trial development.

Metformin is being studied in the National Cancer Institute of Canada Clinical Trails Group's MA.32 trial to determine whether this drug improves invasive disease-free survival of patients with early-stage breast cancer treated with metformin vs placebo in addition to standard adjuvant therapy. Diabetic women are excluded from this trial. Because diabetes increases breast cancer risk and mortality (Coughlin *et al*. 2004, Xue & Michels 2007, Barone *et al*. 2008, Lipscombe *et al*. 2008) and both the diseases are highly prevalent, it would be very helpful to study metformin vs placebo in non-insulin-dependent diabetic women with breast cancer in whom diet and exercise are being used for glucose control. Diabetic women are expected to exhibit a higher risk of recurrence, which would make trials to prevent recurrence through diet, exercise, or pharmacologic intervention easier to perform because of reduced sample size needed for sufficient statistical power. While competing causes of mortality could be an issue, studies of MTOR interception could be performed in younger postmenopausal diabetic women. The studies would answer the question of whether MTOR interception affects breast cancer recurrence in a highly vulnerable population.

Although we know how to define diabetes clinically, self-reporting rather than clinical testing may not be the best approach for the exclusion of patients from trials of metformin or other MTOR-modulating drugs. Just as patients with diabetes may represent a distinct group for study, patients with unidentified diabetes in

a study to prevent breast cancer recurrence may make the study difficult to generalize in all patients. A small subset of unidentified diabetic patients could have a very large benefit, altering interpretation of the results. This type of misclassification bias may become significant because of the high prevalence of both the diseases. In the MA.32 trial, homeostasis model assessment will be applied to estimate insulin sensitivity based on a single measurement of fasting insulin and glucose (Vaccaro *et al*. 2004). Laboratory determination of whether breast cancer patients are insulin resistant, either frankly diabetic or having metabolic syndrome, will greatly help the interpretation of future clinical trials.

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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