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FOR DEBATE

Mitogenic action of insulin: friend, foe or 'frenemy'?

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Abstract Either endogenous or exogenous hyperinsulinaemia in the setting of insulin resistance promotes phosphorylation and activation of farnesyltransferase, a ubiquitous enzyme that farnesylates Ras proteins. Increased availability of farnesylated Ras at the plasma membrane enhances mitogenic responsiveness of cells to various growth factors, thus contributing to progression of cancer and atherosclerosis. This effect is specific to insulin, but is not related to the type of insulin used. The stimulatory effect of hyperinsulinaemia on farnesyltransferase in the presence of insulin resistance represents one potential mechanism responsible for mitogenicity and atherogenicity of insulin.

Keywords Cancer · Farnesyltransferase · Hyperinsulinaemia · Insulin resistance · Mitogenic action

Abbreviations

MAP kinase	Mitogen-activated protein kinase
pCR	Pathological complete response
PDGF	Platelet-derived growth factor
PI3K	Phosphatidylinositol 3-kinase
SOS	Son of sevenless
VEGF	Vascular endothelial growth factor

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Fren-e-my—one who pretends to be a friend but is actually an enemy.

Merriam–Webster Dictionary, 11th edition (1977)

The mere thought that insulin can be detrimental to health has brought chills down the spine to millions of patients with diabetes and their physicians. A hormone that has saved so many lives from the time of its discovery, a hormone that has prevented many diabetic complications, a hormone that is the gold standard of diabetic therapy—this hormone is now suspected to have a negative effect. For so long we have refused to acknowledge this possibility. Even when many epidemiological studies pointed at the association of hyperinsulinaemia with macrovascular complications of diabetes and cancer [1, 2], diabetologists remained unperturbed. With some conciliatory notes on endogenous hyperinsulinaemia, many physicians were unconvinced that exogenous hyperinsulinaemia could be harmful in any other way than the induction of hypoglycaemia.

Suddenly, a few studies in *Diabetologia* [3–5] describing an association between administration of glargine and higher incidence of cancers have aroused worldwide attention. Glargine, a long-acting insulin analogue, is used to mimic basal insulin secretion. Over the last decade, it has become the leading long-acting insulin used either alone or with other glucose-lowering medication and short-acting insulins to provide basal insulinaemia in thousands, if not millions of patients with type 1 and type 2 diabetes. But now these epidemiological studies describing an association between glargine and some forms of cancer have prompted many to re-examine the mitogenic effects of insulin.

The accompanying editorial by Smith and Gale [6] gave an excellent assessment of these studies and the topic in question. Even though these studies implicated glargine, a

larger and fundamentally more important question was whether insulin, endogenous or exogenous, could augment cancer risk in patients with diabetes. Because long-acting insulin analogues do not cause hypoglycaemia in the overwhelming majority of patients, they are used liberally to reduce postabsorptive and pre-prandial hyperglycaemia. In other words, it is easy to be hyperinsulinised while taking glargine or any other long-acting insulin analogues.

Insulin is a major anabolic hormone, which governs carbohydrate metabolism and contributes greatly to the metabolism of lipids and proteins. Clinically, its primary role is to promote glucose utilisation and regulate hepatic glucose production. At the same time, insulin is an important, albeit mild, growth factor. It promotes cell growth and cell division, migration, and also inhibits apoptosis. These aspects of insulin action are collectively known as the 'mitogenic actions' of insulin [7] and because they are so critical to cellular physiology, insulin is always present in cell culture medium for the propagation and maintenance of cells in culture. Although a much weaker mitogen [8] than its cousins, the IGFs, and its more distant relatives such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF) and EGF, insulin has a very specific mitogenic action, which modulates cellular responsiveness to all other growth factors, thus potentiating their actions [9, 10].

Let us now briefly review the molecular mechanisms by which insulin and hyperinsulinaemia, particularly when it occurs in the setting of insulin resistance, can augment proliferative events. In order for all growth factors to stimulate mitogenesis, they must activate the Ras–Raf–mitogen-activated protein (MAP) kinase signalling pathway (Fig. 1). Ras proteins are activated by binding GTP, a process promoted by the guanine nucleotide exchange factor, son of sevenless (SOS). This activation can only occur if Ras proteins are anchored at the plasma membrane (Fig. 1) [11].

Isoprenylation of Ras, as reviewed [12], is the first step that commits Ras to the process of translocation to the

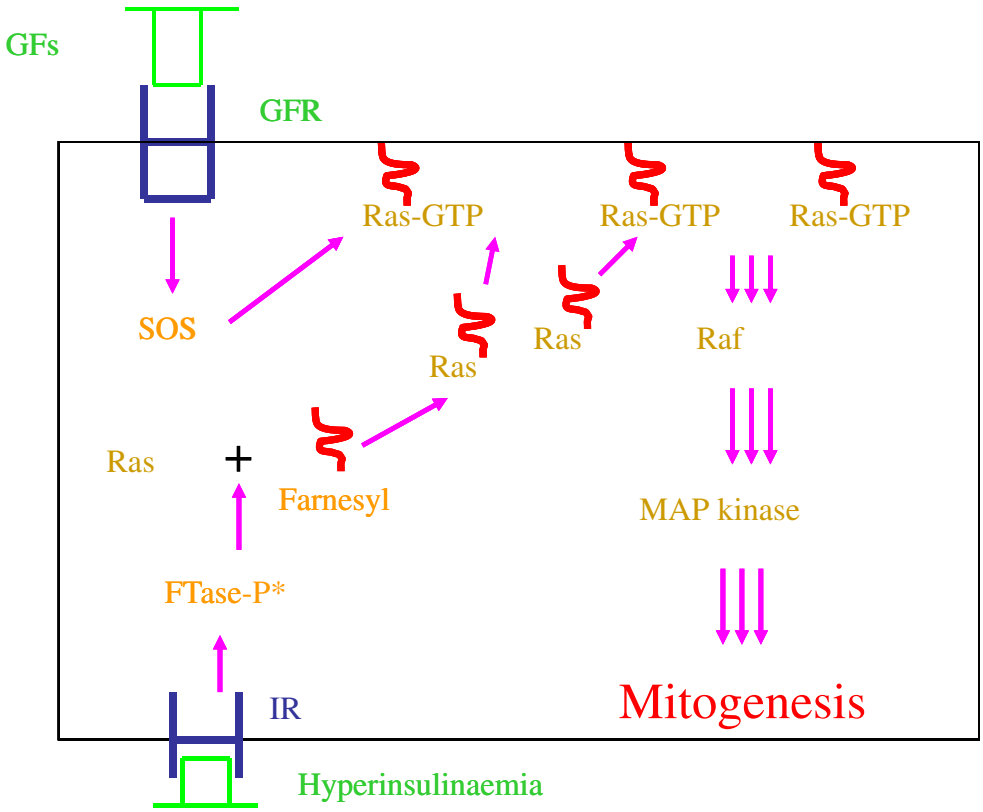

Fig. 1 Insulin potentiates cellular mitogenic responsiveness to growth factors (GFs). GFs (including insulin) interact with their cognate GF receptors and activate the SOS–Ras–Raf–MAP kinase branch of cellular signalling, thus promoting mitogenesis. Initially, GFs stimulate guanine nucleotide exchange factor (SOS), which activates Ras proteins by GTP loading. SOS promotes exchange of GTP for GDP only if Ras is anchored at the plasma membrane. Translocation of Ras protein to the plasma membrane is facilitated by farnesylation of Ras, the attachment of a farnesyl moiety to cysteine residue of Ras under the influence of farnesyltransferase (FTase). Insulin via interaction with its specific cell surface receptor (insulin receptor [IR]) phosphorylates and activates farnesyltransferase, which increases farnesylation of Ras. Hyperinsulinaemia, particularly in the presence of insulin resistance, stimulates farnesyltransferase and augments the amount of farnesylated Ras available for GTP loading in response to other GFs. Greater activation of Ras leads to increased mitogenic responsiveness of cells and tissues, and enhanced mitogenesis and subsequent pathophysiological events

plasma membrane (Fig. 1). Because isoprenylation of Ras involves attachment of a farnesyl moiety (a 15-carbon intermediary in the cholesterol synthesis pathway), the process is also known as farnesylation and is activated by the enzyme farnesyltransferase. Farnesylated Ras is then destined to anchor at the plasma membrane, where it can be activated by growth factors.

As in the case of other growth factors, insulin activates SOS with subsequent activation of farnesylated Ras and other downstream targets. However, unlike other growth factors, insulin also activates farnesyltransferase by phosphorylating its alpha subunit [13, 14]. Phosphorylation and activation of farnesyltransferase increases the amounts of membrane-bound, farnesylated Ras available for activation by other growth factors (Fig. 1) [15]. This effect of insulin on farnesyltransferase is specific for insulin and not mimicked by other growth factors [9]. Moreover, activation of farnesyltransferase by insulin requires an intact insulin receptor, but not IGF-1 receptor, indicating that this action is mediated exclusively by the insulin receptor and is not an ancillary effect of interaction between insulin and IGF-1 receptors. This was demonstrated in cells expressing the chimeric insulin/IGF-1 receptor and in cells derived from insulin receptor knockout animals [9]. Furthermore, in the context of insulin resistance, where the canonical phosphatidylinositol 3-kinase (PI3K)/Akt metabolic pathway of insulin signalling is inhibited to various degrees, the Ras–Raf–MAP kinase mitogenic pathway of insulin is undisturbed and possibly upregulated, leading to increased insulin-stimulated activation of farnesyltransferase with subsequent increases in the amounts of farnesylated Ras [14, 15]. Taken together, these actions of insulin, although normal within the context of insulin signalling, enhance the mitogenic responsiveness of cells and tissues.

The crux of the matter is that hyperinsulinaemia, whether in cell culture or in vivo (i.e. in animals and humans), leads to overstimulation of farnesyltransferase, and excessive farnesylation and membrane association of Ras proteins, thereby increasing cellular responsiveness to other growth factors. This potentiation of the mitogenic effects of other growth factors becomes critical in the pathophysiology of progression of cancer and vascular disease [15–17].

Several in vivo studies have provided observational and experimental support for this hypothesis. Thus, liver, aorta and skeletal muscle of insulin-resistant *ob/ob* mice and *fa/fa* rats contained increased amounts of farnesylated Ras [18]. Reduction of hyperinsulinaemia by exercise training resulted in decreased amounts of farnesylated Ras in Zucker *fa/fa* rats [18]. Induction of fetal hyperinsulinaemia by direct infusion of insulin into the fetus and by fetal or maternal infusions of glucose resulted in significant increases in the activity of farnesyltransferase and the amounts of farnesylated Ras in fetal liver, skeletal muscle, fat and white blood cells [19]. An additional infusion of somatostatin into hyperinsulinaemic fetuses blocked fetal hyperinsulinaemia and completely prevented these increases, specifying insulin as a causative factor. In other studies, insulin infusions significantly increased the amounts of farnesylated Ras in white blood cells of humans, in liver samples from mice and dogs, and in aorta samples of mice [10]. Taken together, these findings strongly support the in vivo relationship between insulin resistance and the ability of insulin to stimulate farnesyltransferase activity.

Overall, the ability of insulin to potentiate action of IGF-1, EGF, PDGF and VEGF has been observed in a variety of tissues, including vascular smooth muscle cells, endothelial cells, adipocytes, fibroblasts, liver cells and breast cancer cells [9, 10, 13, 15–21]. This effect of insulin has been consistently observed whenever metabolic insulin resistance along the PI3K pathway is present. Thus, enhanced cellular responsiveness to growth factors is a physiological effect of insulin that becomes pathological in response to hyperinsulinaemia, whether endogenous (secondary to insulin resistance) or exogenous (secondary to chronic iatrogenic overinsulinisation of insulin-resistant individuals).

The question that lies before us is: what are the clinical implications of the hypothesis that hyperinsulinaemia, in a setting of insulin resistance, exerts significant mitogenic and pro-atherogenic influence? Clearly, acceptance of this postulate would demand an aggressive correction of insulin resistance in order to diminish endogenous compensatory hyperinsulinaemia and to minimise exogenous hyperinsulinaemia. The most appropriate way of addressing insulin resistance therapeutically is to implement lifestyle modifications, i.e. diet and physical activity. Dietary compliance must return to its proper place as a cornerstone of diabetes therapy [22], while the practice of compensating for dietary indiscretions with increasing doses of insulin should be discouraged.

Most patients with type 2 diabetes are insulin-resistant. For them, paying ‘lip service’ to dietary and lifestyle therapies leads to overinsulinisation. Many patients with type 1 diabetes who use large doses of insulin to cover for their excessive intake of carbohydrates are also insulin-resistant. Carbohydrate counting is an extremely useful tool in diabetes therapy, but consuming unlimited amounts of dietary carbohydrate also leads to overinsulinisation. A second-best approach to improving insulin resistance would be the use of insulin sensitisers such as metformin.

Several studies have demonstrated an increased likelihood of developing cancer in patients treated with insulin or insulin secretagogues as compared with metformin [4, 23]. Data collected between 1991 and 1996 demonstrated that patients with type 2 diabetes mellitus exposed to sulfonylureas and exogenous insulin had significantly

greater cancer-related mortality rates than did patients treated with metformin [23]. Recently, Jiralerspong and colleagues [24] compared the rates of pathological complete response (pCR) in diabetic patients with breast cancer who were receiving neoadjuvant chemotherapy and being treated with metformin or not. The rate of pCR (better outcomes) was 24% in the metformin group and 8% in the non-metformin group ($p=0.007$). While the use of insulin did not influence the rate of pCR in the metformin-treated group, its use in the non-metformin group was associated with the lowest rate of pCR ($p=0.05$).

Undoubtedly, insulin (human, pork, beef and analogues) as such does not cause cancer or atherosclerosis. If anything, insulin, a life-saving hormone, has dramatically improved the life expectancy of patients with diabetes. However, high physiological concentrations of insulin can substantially increase cellular mitogenic responsiveness to other growth factors and promote disadvantageous growth and proliferation, particularly in the presence of insulin resistance.

The constellation of metabolic insulin resistance (diminished strength of insulin signalling along the PI3K branch of its action) and hyperinsulinaemia results in overstimulation of the MAP kinase signalling branch and chronic activation of farnesyltransferase, with subsequent increases in the amounts of farnesylated Ras. These events augment the mitogenicity of other growth factors, thereby promoting the progression of cancer and atherosclerosis.

Even though the number of glucose-lowering medications has increased dramatically in the last decade, it is important that attention not be distracted from the underlying deleterious effects of insulin resistance, which play key roles in redirecting insulin signalling to strengthen the mitogenic branch of insulin action.

Even though activation of farnesyltransferase by hyperinsulinaemia in the presence of insulin resistance could be responsible for mitogenicity in these patients, it will be important in the future to evaluate the effect of farnesyltransferase inhibitors on prevention and delay of progression of cancer and atherosclerosis in animal models of insulin resistance and eventually in humans. Equally important will be the clarification of whether improvements in insulin sensitivity, achieved by lifestyle modifications or insulin sensitisers, can lower the risk of cancer and atherosclerosis in insulin-resistant populations.

In summary, the detrimental mitogenic effects of hyperinsulinaemia must be addressed along with hyperglycaemia when treating diabetes. Endogenous hyperinsulinaemia must be treated by minimising insulin resistance with diet, exercise and insulin-sensitising medications, whereas exogenous hyperinsulinaemia must be avoided by selecting appropriate diet and lifestyle, while using minimal doses of insulin to achieve normoglycaemia. Inducing hyperinsulinaemia as a price for paying 'lip service' to dietary therapy is not only inexcusable, but also potentially harmful. Hence, insulin, the 'best friend' of patients with diabetes, could become a 'frenemy' if used in excess in the setting of insulin resistance.

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