

# Insulin Analogues with Increased Mitogenic Potency – Are they Safe?

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## Abbreviations

▼	
HMEC	Human Mammary Epithelial Cells
IGF1	Insulin-like growth factor-1
IGF1R	Insulin-like growth factor-1 receptor
Insulin Aspart	B28Asp human insulin
Insulin Detemir	B29Lys( $\epsilon$ -tetradecanoyl), desB30 human insulin
Insulin Glargine	A21Gly,B31Arg,B32Arg human insulin
Insulin Glulisine	B3Lys,B29Glu human insulin
Insulin LisPro	B28Lys,B29Pro human insulin
IR	Insulin receptor

Insulin analogues have been and are still being developed by modifying the insulin molecule in such a way that the injected insulin analogues mimic the pharmacokinetics of endogenously produced insulin in the best possible way. Two approaches have been pursued: site-directed mutagenesis and attachment of fatty acid molecules by acylation to produce insulin analogues with faster action (insulin LisPro, Aspart, and Glulisine) or with a more prolonged action (insulin Glargine and Detemir). These analogues have lead to improved blood glucose regulation and greatly improved the convenience for the patients [1]. Insulin has, besides its well known metabolic actions, effects related to growth and gene-expression and can be shown to have mitogenic effects in various cell systems [2]. Given the life long exposure and the patient population involved, it is of utmost importance to ensure that new insulin analogues are free of carcinogenic effects. Potential risks with new insulin analogues can be summarized as follows:

► Increased mitogenesis could, from a theoretical point of view, lead to an increased risk of mutagenesis purely based upon statistics;

- The modified insulin analogue could be mutagenic in itself leading to an increased rate of mutagenic occurrences,
- Already transformed cells could be more sensitive to growth promoting effects of modified insulin analogues due to changes in receptor expression and/or distribution (i.e., the proportion between IR and IGF1R), and
- Increased mitogenic potency *per se* could induce unwanted growth effects, such as atherogenic effects.

Of these potential risks, growth of already transformed cells is considered to be the most hazardous by the European Agency for the Evaluation of Medicinal Products (EMA). To test modified insulin analogues for possible increased carcinogenic potential in standard toxicological investigations, experimental animals are exposed to high doses of insulin analogues for several months. Unfortunately, insulin and insulin analogues by nature reduce blood glucose and studies with high doses of insulin or insulin analogues often lead to severe hypoglycemic events, which makes these studies quite difficult to perform and the results are somewhat difficult to interpret. There are also the secondary effects of long-term insulin treatment such as increased weight gain that may contribute indirectly to any observed carcinogenicity, thus confounding conclusions.

Therefore, studies measuring the mitogenic potential of insulin analogues on a cellular level have been widely used to supplement long-term toxicology studies, acting as a surrogate assay to monitor the potential risk of insulin analogues. However, it is important to point out that increased mitogenicity in itself does not necessarily lead to increased carcinogenicity.

The discussion regarding the role of the mitogenicity of insulin analogues was fueled by the rather surprising finding that insulin X10 with a *single* amino acid substitution (B10D) in a

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**Table 1** Studies of insulin Glargine

Cell types	IGF1R:IR Ratio	IGF1R Affinity	Mitogenicity	Ref.
MCF-7, SKBR-3, Saos-2	7:1, 1:1, and 10:1	nd	↔↑	[6]
Rat-1 overexpressing IR	Predominantly IR	nd	↔	[11]
MCF-7, MCF-10	4:1 and 1:1	nd	↔	[8]
H9c2 cardiomyocytes	Predominantly IGF1R	↑	↔	[12]
HMEC	Predominantly IGF1R	↑	↑	[13]
Cultured human skeletal muscle cells	nd	↑	↔	[14]
SaosB10	Predominantly IGF1R	↑	↑	[4]

dose-dependent manner increased the incidence of adenocarcinomas and fibroepithelial tumors in female Sprague Dawley rats [3]. At the same time X10 was shown to demonstrate several characteristics different from human insulin: increased insulin receptor affinity (2 to 3-fold), increased affinity for the IGF1R (7 to 10-fold), decreased receptor dissociation rate (7-fold) and sustained signaling from the receptor [4,5].

Insulin Glargine has received special attention (including the recent paper by Liefvendahl et al. in this journal [6]) since changes in the C-terminus of the insulin molecule can increase the affinity for the IGF1R and might induce an increased mitogenic potency [7] and an increased affinity for the IGF1R was indeed found in several studies (Table 1).

Most reports indicate that insulin Glargine binds to IR with a slightly lower potency than human insulin, but with a 2 to 7-fold higher affinity to IGF1R. However, there seems to be some controversy with respect to the relative mitogenic potency of insulin Glargine (Table 1). In several cell types insulin Glargine was found to be comparable to human insulin in stimulating cell division. In contrast, there is clearly a higher mitogenic potency of insulin Glargine compared to human insulin in HMEC (Human Mammary Epithelial Cells) and SaosB10 cells. The reason for this discrepancy cannot be attributed to the selection of transformed versus nontransformed cells, since HMEC is a primary nontransformed cell line and SaosB10 is an Osteosarcoma cell line. The proportion of IR to IGF1R may explain at least partly the observed differences, since increased mitogenic potency of insulin Glargine is only seen in cells which have a high proportion of IGF1R. Nevertheless, Staiger et al. [8] failed to detect any increased mitogenic potency of insulin Glargine in MCF-7 cells, despite the fact that they express 4-fold more IGF-1R receptors than IR. This suggests that a certain degree of IGF1 to insulin receptors ratio is needed before a mitogenic response to insulin Glargine can be observed.

However, it should be noted that measuring mitogenicity in some cell types can be quite difficult and laborious due to a relatively poor response and large variability. When only a 2-fold stimulation of mitogenicity is observed in response to a maximally stimulating concentration and/or only a few concentrations are used, a precise estimate of ED<sub>50</sub> values can be difficult to make. As a consequence, in these situations it may not be possible to demonstrate a significant difference in mitogenic potencies.

A careful examination of the data underlying Table 1 reveals that in general whenever there is a relatively high mitogenic response as well as a high proportion of IGF-1Rs, insulin Glargine proves to be more mitogenic than human insulin. This is especially evident in the work of Liefvendahl et al. where 3 different cell types were used in the same study. The SaosB2 cells showed a solid mitogenic response, and accordingly, it is only in these cells that the response to IGF1 is clearly different from insulin and that

insulin Glargine tends to have an increased mitogenic potency [6]. This conclusion fits nicely with observations that in cells expressing IGF1R there is a good correlation between the insulin analogues' affinity for the IGF1R and the ability to stimulate mitogenicity [4,7].

Thus, it seems safe to conclude that insulin analogues with an increased affinity for the IGF1R (such as insulin Glargine) are more mitogenic than human insulin when tested in cells expressing a high proportion of IGF1R.

However, the question remains whether this has any clinical implication. Several uncertainties make this question very difficult to answer decisively. First of all, the circulating concentrations of injected insulin analogues are normally quite low compared to the levels needed to elicit a mitogenic response *in vitro*. On the other hand these concentrations (low nanomolar) are able to elicit *in vitro* metabolic responses.

Moreover, since insulin X10 was found to induce growth of tumors in rat mammary tissue, it can, in accordance with the above considerations, be speculated that insulin X10 either induces mutagenic events *per se* leading to transformation or results in an increased growth rate of spontaneously occurring malignant cells. The explanation for this is unknown, but has been correlated to the increased mitogenic potency, which again has been attributed to the increased affinity for IGF1R [4,7] or sustained signaling from the insulin receptor [5].

A toxicological study (2–12.5 IU/kg) with insulin Glargine revealed no occurrences of mammary gland tumors and no increase in transforming ability [9]. In addition, insulin Glargine has by now been administered to a large number of people with diabetes for several years without any reported correlation to an increased incidence of tumorigenesis. Therefore, it seems that an increased affinity for IGF1R does not *per se* result in increased risk of tumorigenesis. This may then, in turn, point more towards the idea that sustained signaling from the receptor could be responsible. This is, however, difficult to conclude at the present time since only one example of an insulin analogue (X10) with an increased tumorigenic potential compared to human insulin has been identified. Thus, the direct link between sustained signaling and increased tumorigenicity is still currently lacking.

Furthermore, the evidence for the correlation between increased mitogenicity and increased risk of carcinogenesis rests solely on a single example where mammary tumors were observed in SD rats after treatment with high doses of insulin X10. Could this have been a random occurrence? Perhaps, since in a separate 52-week rat study, human insulin was capable of inducing mammary gland tumors when given at a similarly high dose. Thus, there is little clear evidence to link increased mitogenicity of insulin analogues to increased risk of carcinogenesis.

Finally, it is known that insulin Glargine, upon injection, is processed by enzymatic removal of the two C-terminal arginines on

the B-Chain, resulting in insulin A21Gly [10], which has both a low IGF1R affinity and a low mitogenic potency [4]. If this occurs to a large extent *in vivo* it might explain why insulin Glargine, despite an increased IGF1R affinity and an increased mitogenic potency *in vitro*, does not result in any increased risk of tumorigenesis. This latter possibility taken together with the risk of undesired nonmalignant growth effects speaks strongly towards recommending that future insulin analogues should be developed free of any disproportionate increased mitogenic potency.

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### Erratum

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An error occurred in the author's list in the article mentioned above. The correct list of authors is as follows:

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