# 读书报告

汇报人：邓大鹏

时　间：2019.6.16

# Mechanisms Underlying the Synergistic Action of Insulin and Growth Hormone on IGF-I and -II Expression in Grass Carp Hepatocytes

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IF=3.519

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In mammals, insulin is known to modify growth hormone (GH)-induced IGF-I expression at the hepatic level, which also contributes to the functional crosstalk between energy homeostasis and somatotropic axis. However, the studies on the comparative aspects of this phenomenon are limited and the mechanisms involved have not been fully characterized. Using a serum-free culture of grass carp hepatoctyes, the functional interaction between GH and insulin on hepatic expression of IGF-I and -II was examined in a fish model. In carp hepatocytes, GH could up-regulate IGF-I and -II mRNA expression

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# •研究背景

在哺乳动物中，胰岛素样生长因子(IGF)有两种形式，即IGF-I和IGF-II。IGF-I在生长轴介导的产后生长中起着关键作用，其表达受生长激素GH的调控，而IGF-II的表达不受GH的影响。在鱼类中，肝脏IGF-I和IGF-II产生/分泌均受GH的调控。

有研究表明，在鸡和老鼠的肝细胞中，insulin可增强GH诱导的IGF的表达，但作用机制尚不清楚，而在鱼类中类似研究得出的结果确是可变的。因此，本文以草鱼为研究对象，旨在探究insulin和GH对草鱼肝细胞中IGF表达的协同作用机制。

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01 研究背景

02 材料与方法

03 结果与分析

04 结论

缩写：

GH:生长激素；

IGF:胰岛素样生长因子；

GHR:GH受体；

Ins R:胰岛素受体；

IGF1R:IGF-I受体；

MAPK:有丝分裂原活化蛋白激酶；

MEK ½:有丝分裂原活化蛋白激酶1/2；

ERK ½:细胞外信号调节激酶；

P38 MAPK:P38有丝分裂原活化蛋白激酶；

PI3K:磷酸肌醇3-激酶；

Akt:蛋白激酶B；

mTOR:雷帕霉素靶蛋白；

JAK 2:Janus激酶2；

STAT 5:信号转导和转录激活因子。

1.以草鱼(1.8-2.1kg)为研究对象，分别用GH(10-1000ng/ml)和insulin(0.01-100nM)孵育肝细胞24h，测定IGF的表达量。

2.分别用GH(300ng/ml)，insulin(10nM)与JAK2/STAT5，$MEK_{1/2}$/$ERK_{1/2}$，PI3K/Akt/mTOR，P38 MAPK通路上关键酶的抑制剂共同孵育肝细胞24h，测定IGF的表达量, 并测定$MEK_{1/2}$，$ERK_{1/2}$，Akt，P38 MAPK的磷酸化水平。

3.分别用GH(300ng/ml)，insulin(10nM)以及GH(300ng/ml)和insulin(10nM)共同孵育肝细胞6，12，24h，测定IGF的表达量；用GH(300ng/ml)和insulin(0.01-100nM)共同孵育肝细胞24h，insulin(10nM)和GH(10-1000ng/ml)共同孵育肝细胞24h，测定IGF的表达量。

4.用insulin(10nM)孵育肝细胞15min，测定InsR的磷酸化水平；分别用GH(300ng/ml)，insulin(10nM)以及GH(300ng/ml)和insulin(10nM)与InsR激活阻断剂HNMPA(10μM) 共同孵育肝细胞24h，测定IGF的表达量。

5.分别用GH(300ng/ml)，insulin(10nM)以及GH(300ng/ml)和insulin(10nM)共同孵育肝细胞0，30，60，90，120min，测定InsR，STAT5，Akt和 $ERK_{1/2}$的磷酸化水平。

6.分别用GH(300ng/ml)，insulin(10nM)或GH(300ng/ml)和insulin(10nM)孵育肝细胞15min，从肝细胞中制备膜蛋白，用免疫沉淀法检测InsR或GHR。

7.分别用GH(300ng/ml)，insulin(10nM)或GH(300ng/ml)和insulin(10nM)与JAK2/STAT5，$MEK_{1/2}$/$ERK_{1/2}$，PI3K/Akt/mTOR，P38 MAPK关键酶抑制剂共同孵育肝细胞24h，测定IGF的表达量。

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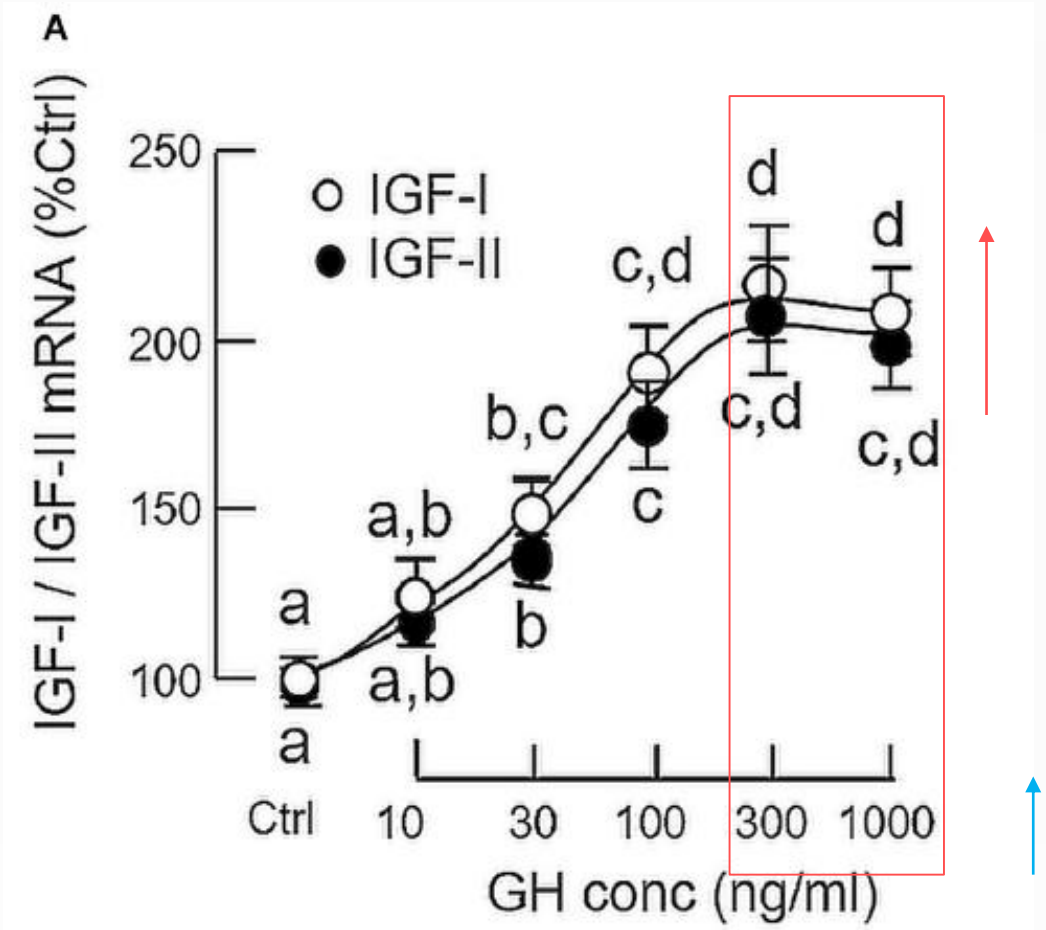
# •GH对IGF基因表达的调控作用

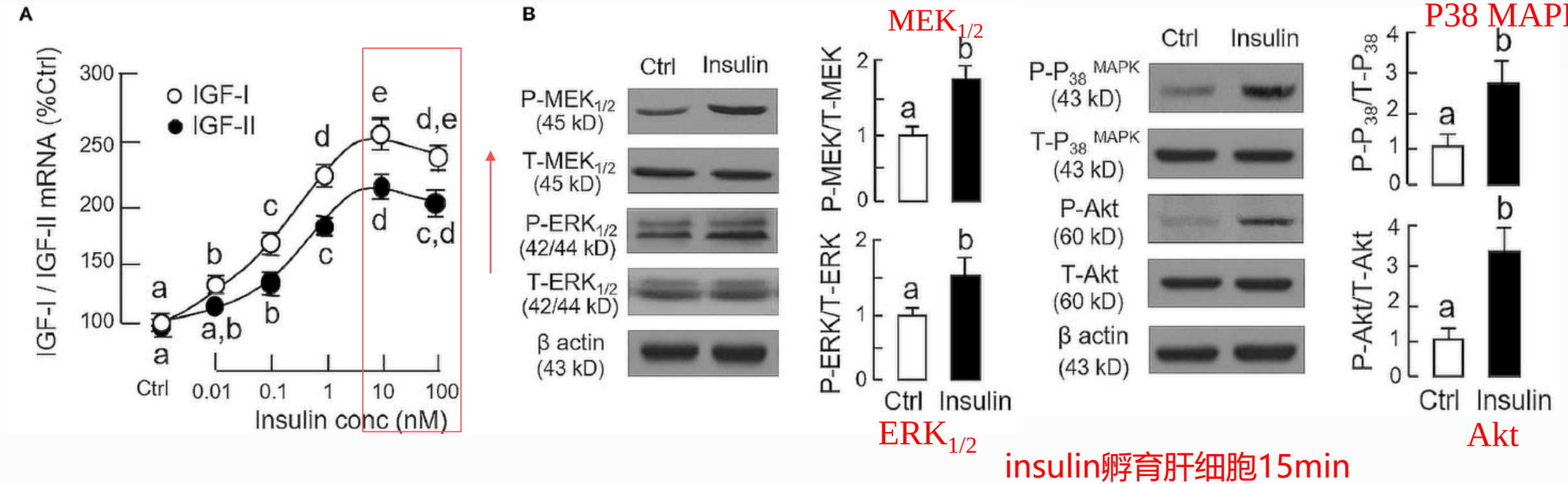



B

| Drug Treatment (Ctrl as 100%) | IGF-I mRNA (%Ctrl) | IGF-II mRNA (%Ctrl) |
|---|---|---|
| GH alone | 212.1 ± 15.3 | 205.7 ± 9.2 |
| JAK2 STAT5 GH + AG490 | 77.5 ± 12.4 * | 61.1 ± 12.0 * |
| GH + NICO | 74.2 ± 14.6 * | 68.2 ± 15.2 * |
| PI3K Akt mTOR GH + LY294004 | 73.1 ± 25.7 * | 65.4 ± 18.7 * |
| GH + HIMOC | 85.1 ± 22.0 * | 86.5 ± 20.7 * |
| GH + Rapamycin | 90.2 ± 19.6 * | 88.7 ± 18.2 * |
| $MEK_{1/2}$ $ERK_{1/2}$ GH + PD98059 | 67.2 ± 20.8 * | 70.7 ± 23.2 * |
| GH + FR180204 | 71.2 ± 18.6 * | 61.7 ± 14.2 * |
| P38 MAPK GH + SB203580 | 375.2 ± 24.1 * | 366.4 ± 27.0 * |

图1 (A) Effects of increasing doses of GH treatment (10–1,000 ng/ml) on IGF-I and -II mRNA expression. (B) JAK2/STAT5, MAPK, and PI3K/Akt pathways in IGF-I and -II mRNA expression induced by GH.

# •insulin对IGF基因表达的调控作用




insulin孵育肝细胞15min

图2 (A) Effects of increasing doses of insulin treatment (0.01–100 nM) on IGF-I and -II mRNA expression. (B) Insulin stimulation on protein phosphorylation of $MEK_{1/2}$ , $ERK_{1/2}$ , P38 MAPK, and Akt in carp hepatocytes.

C

| Drug Treatment (Ctrl as 100%) | IGF-I mRNA (%Ctrl) | IGF-II mRNA (%Ctrl) |
|---|---|---|
| Insulin alone | 225.2 ± 22.3 | 208.7 ± 26.2 |
| Insulin + LY294004 | 75.4 ± 25.7 * | 66.4 ± 18.7 * |
| Insulin + HIMOC | 113.9 ± 23.0 * | 105.5 ± 20.7 * |
| Insulin + Rapamycin | 93.2 ± 19.6 * | 108.7 ± 21.1 * |
| Insulin + PD98059 | 211.1 ± 24.8 | 208.7 ± 26.3 |
| Insulin + FR180204 | 219.3 ± 27.6 | 221.5 ± 24.7 |
| Insulin + SB203580 | 391.7 ± 30.2 * | 404.4 ± 37.0 * |
| Insulin + AG490 | 84.1 ± 20.4 * | 96.2 ± 17.3 * |
| Insulin + NICO | 116.1 ± 18.8 * | 108.6 ± 21.1 * |

PI3K
Akt
mTOR
$MEK_{1/2}$
$ERK_{1/2}$
P38 MAPK
JAK2
STAT5

图2 (C) PI3K/Akt, P38 MAPK, and JAK 2/STAT 5 pathways in IGF-I and -II mRNA expression induced by insulin.(孵育24h)

# •GH和insulin相互作用对IGF基因表达的影响

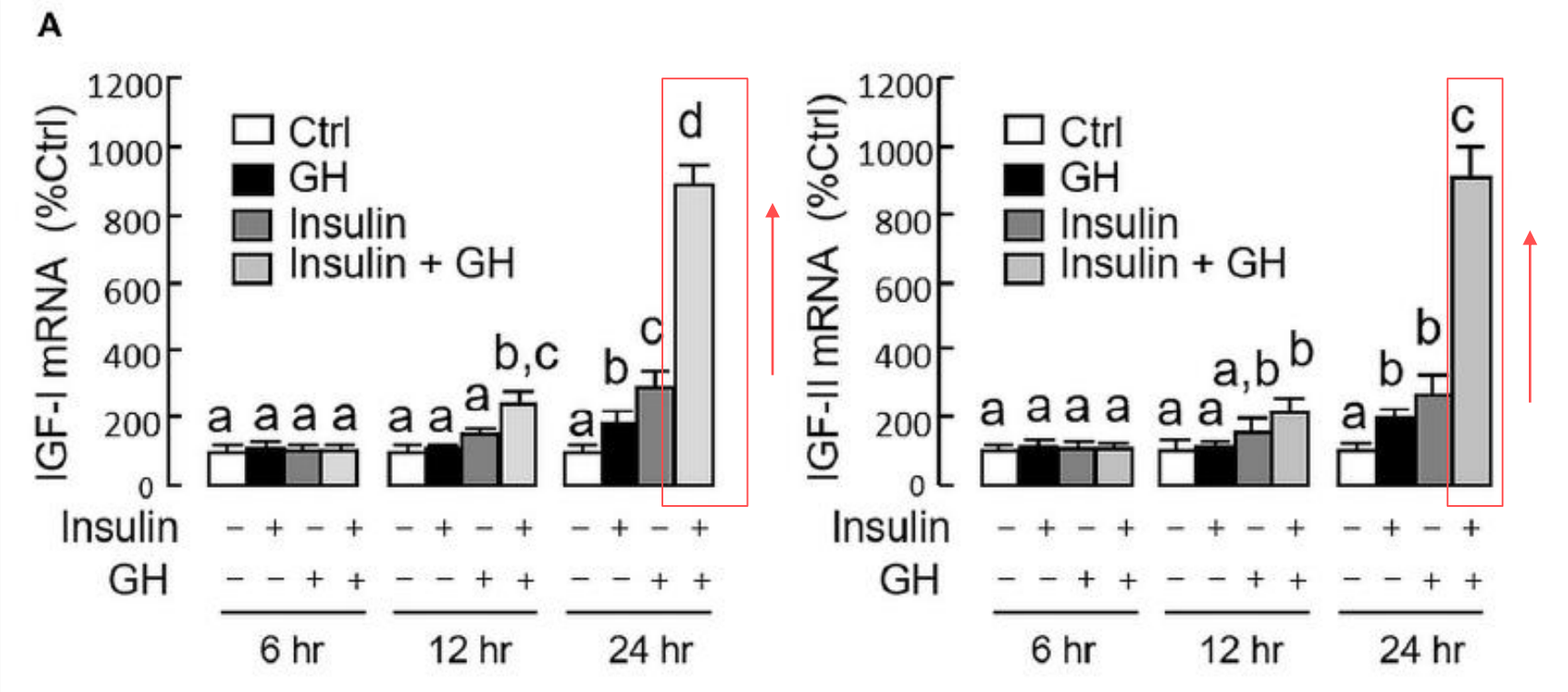



图3 (A) Time course of GH alone (300 ng/ml), insulin alone (10 nM) and co-treatment with GH (300 ng/ml) and insulin (10 nM) on IGF-I and -II m RNA expression

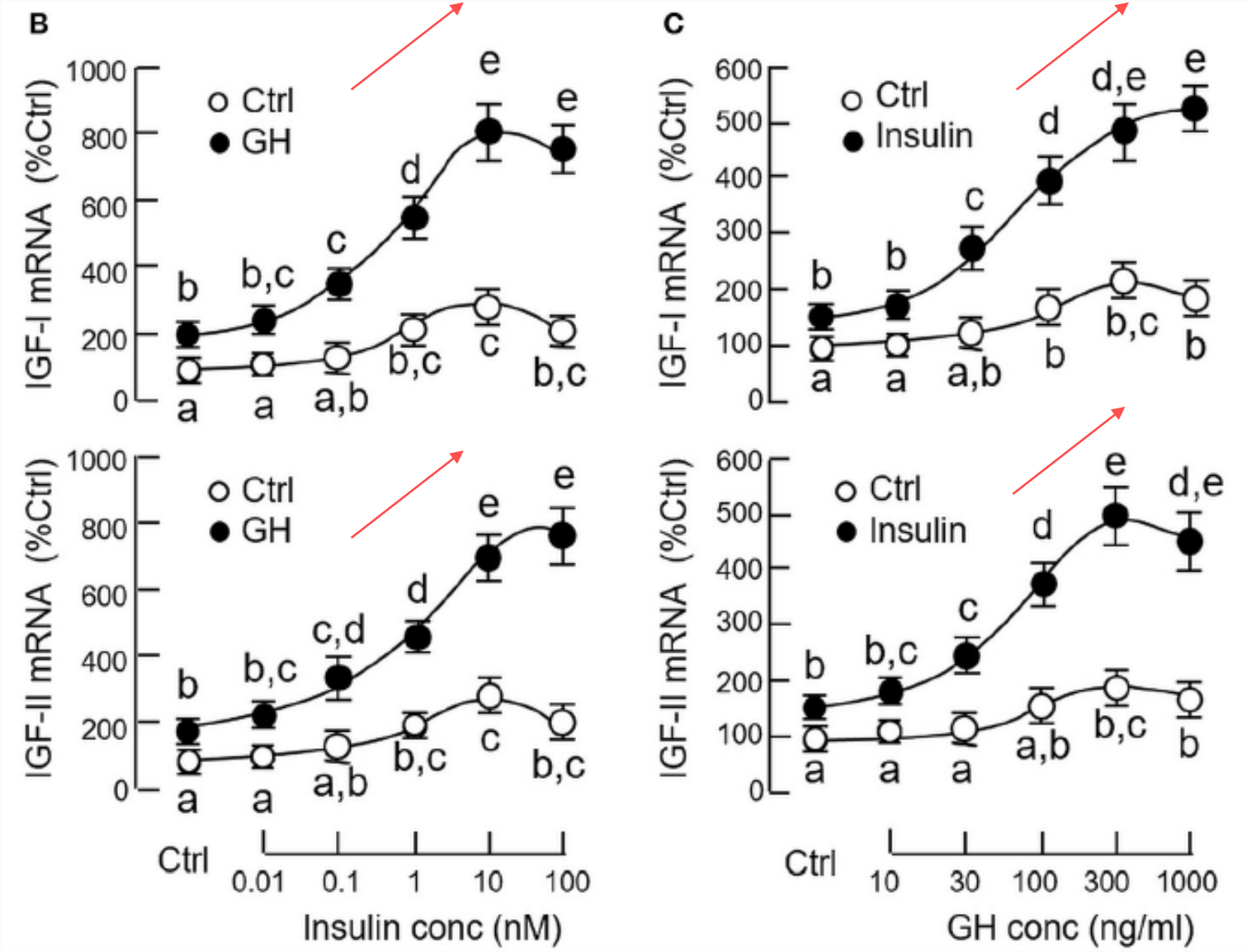



图3 (B) Dose dependence of increasing levels of insulin (0.01–100nM) on IGF-I and -II mRNA expression induced by GH treatment (300ng/ml). (C) As a reciprocal experiment, increasing levels of GH treatment (10–1000ng/ml) on IGF-I and -II mRNA expression induced by insulin (10nM) was also tested in carp hepatocytes. In dose-response studies, the duration of drug treatment was fixed at 24 h. 孵育24h

GH和insulin的相互作用对IGF的表达存在剂量性依赖。

## Insulin受体特异性

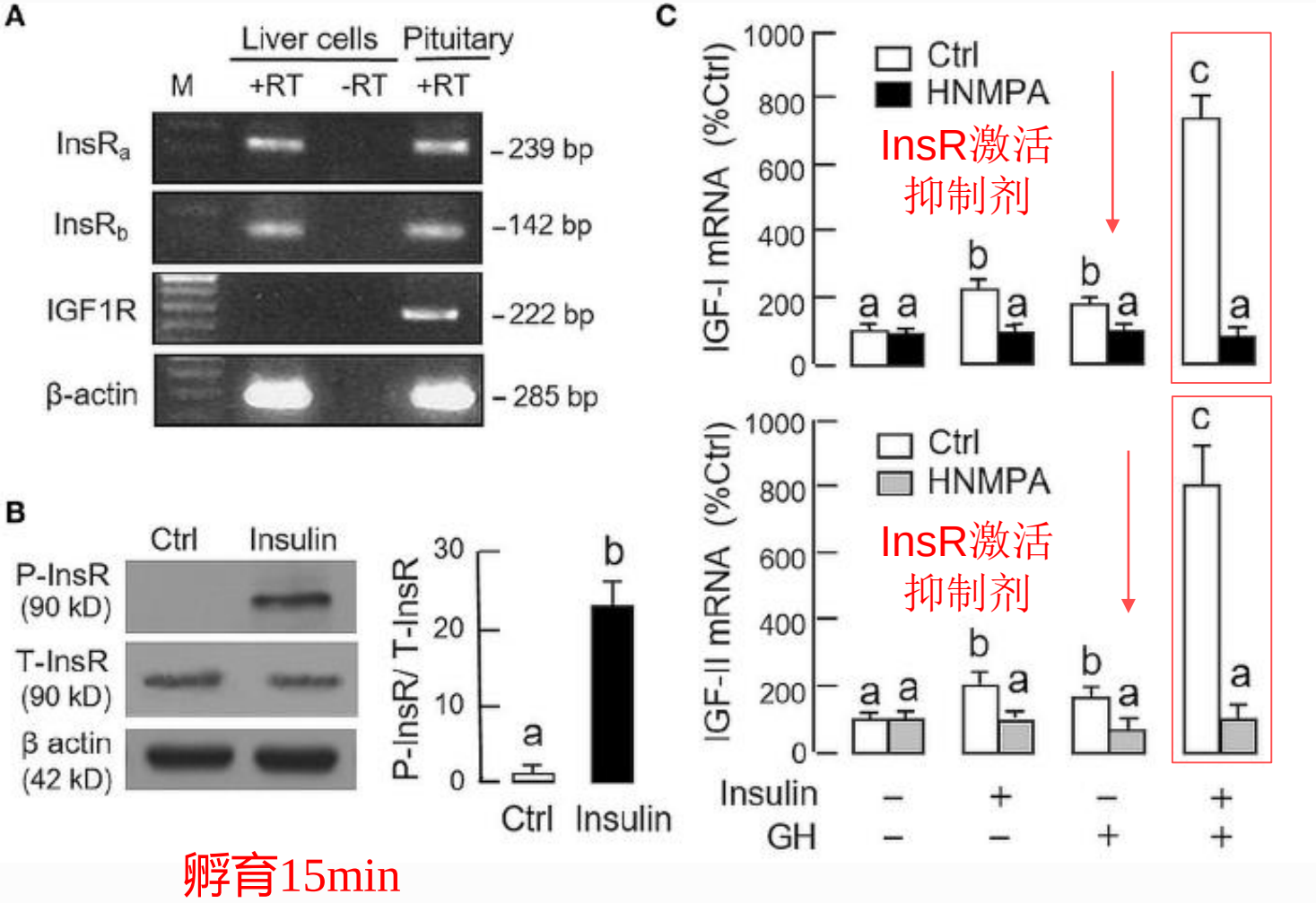



图4 Receptor specificity for insulin potentiation of GH-induced IGF-I and -II expression in carp hepatocytes. (A) RT-PCR for Ins Ra , Ins Rb , and IGF1R expression in carp hepatocytes (B) Insulin treatment on protein phosphorylation of Ins R in carp hepatocytes. (C) Blockade of Ins R activation on insulin potentiation of GH-induced IGF-I and -II mRNA expression in carp hepatocytes. Hepatocytes were incubated for 24 h with GH alone (300 ng/ml), insulin alone (10 nM) or co-treatment of GH (300 ng/ml) and insulin (10 nM) in the presence of HNMPA (10 μM), an inhibitor for Ins R activation.

孵育15min

孵育24h

GH和insulin的协同作用是通过激活InsR来增强IGF的表达

# •GH和insulin对IGF基因表达的协同作用机制

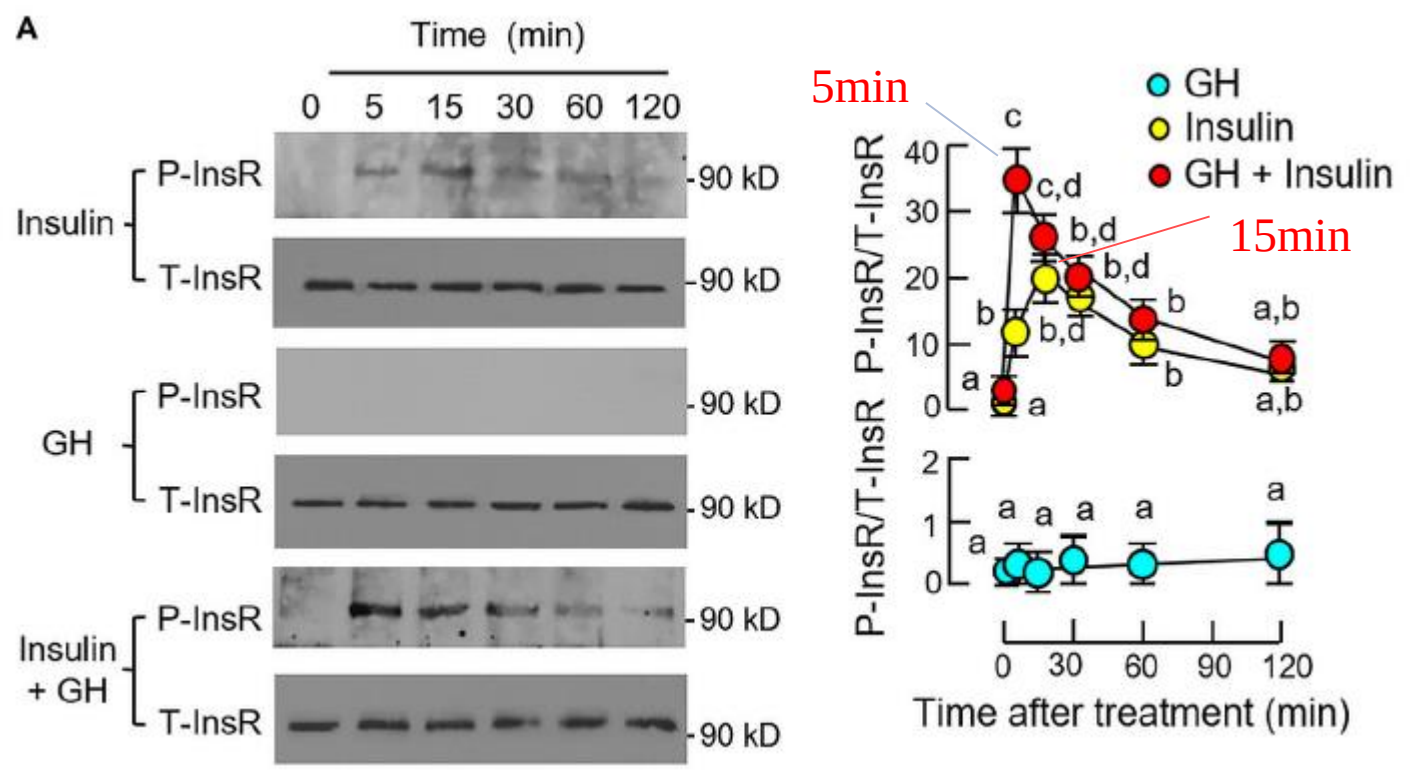



图5 Synergistic effect of GH and insulin on Ins R, STAT 5 , Akt, and $ERK_{1/2}$ activation in carp hepatocytes. (A) GH enhancement of Ins R phosphorylation induced by insulin treatment.

GH可增强 insulin-induced InsR的磷酸化

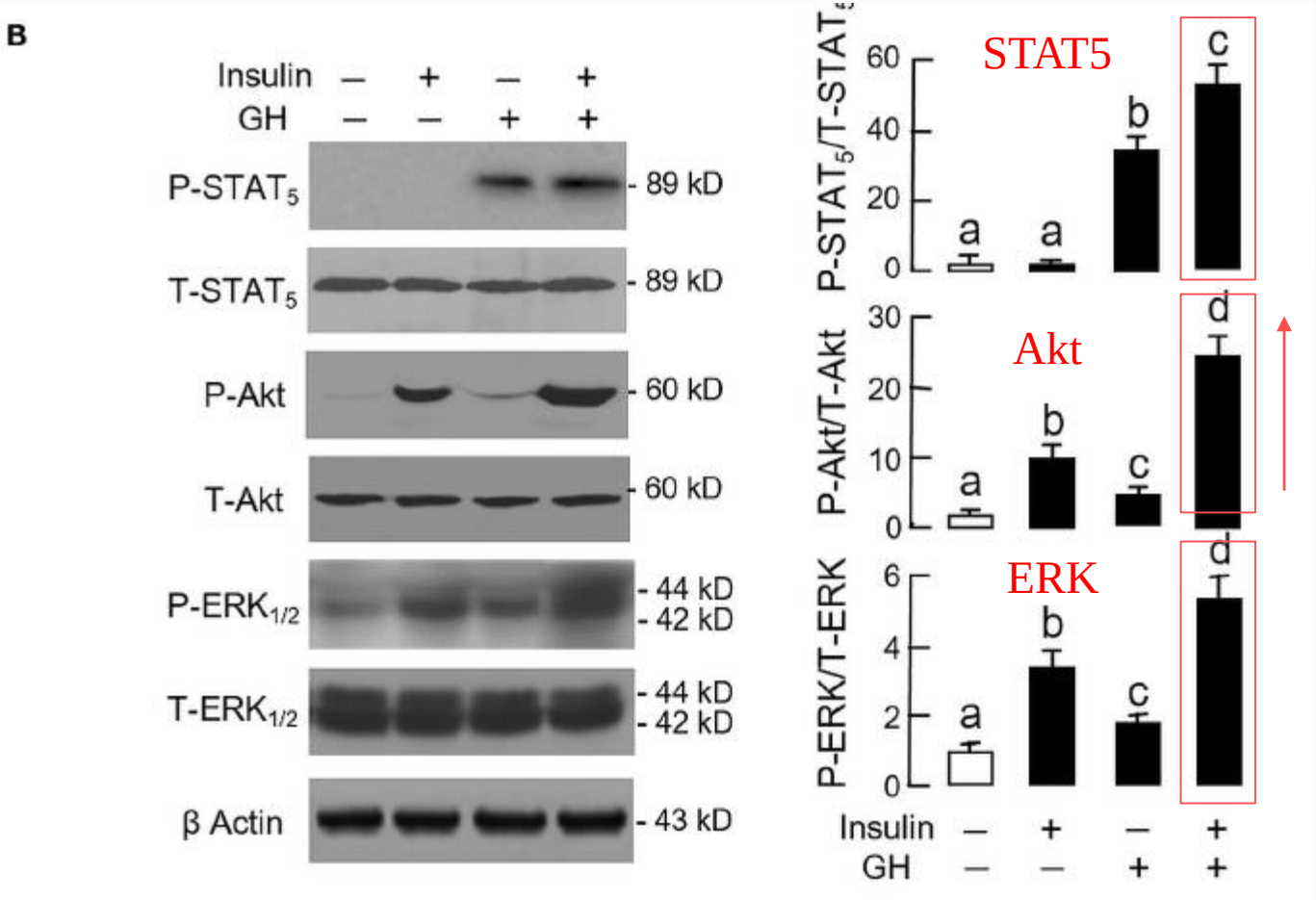



图5 Synergistic effect of GH and insulin on Ins R, STAT 5 , Akt, and $ERK_{1/2}$ activation in carp hepatocytes. (B) Insulin potentiation of STAT 5 , Akt, and $ERK_{1/2}$ phosphorylation induced by GH treatment. In these experiments, hepatocytes were exposed to insulin alone (10 nM), GH alone (300 ng/ml) or co-treatment of GH (300 ng/ml) and insulin (10 nM) for the duration as indicated for Ins R phosphorylation.

孵育0-120min

GH和insulin的协同作用可增强STAT5，Akt和$ERK_{1/2}$酶磷酸化。

那么，是什么原因引起信号通路关键酶磷酸化增强的呢？膜受体的相互结合可改变各自受体后的信号传导。

IP:免疫沉淀

IB:蛋白质印迹

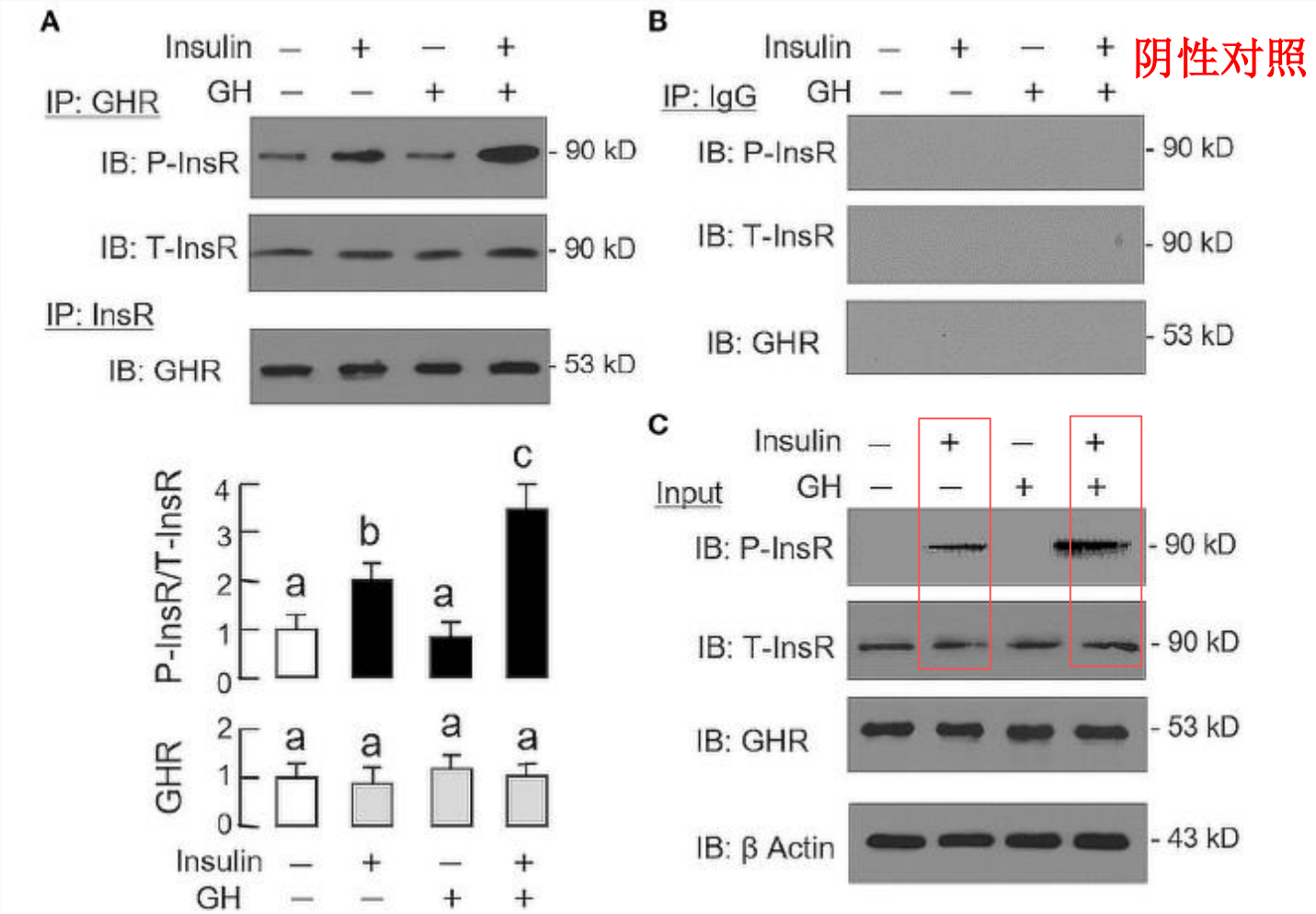



**FIGURE 6 |** Protein:protein interaction of GHR and InsR expressed in carp hepatocytes. Membrane protein extract was prepared from hepatocyte after challenged for 15-min with insulin alone (10 nM), GH alone (300 ng/ml) or co-treatment with GH (300 ng/ml) and insulin (10 nM) and used in immunoprecipitation (IP) with antibody for GHR or total protein of InsR **(A)**. For protein samples pulled down by IP against GHR, size-fractionation by SDS-PAGE was conducted followed by immunoblotting (IB) with antibodies for phosphorylated form (P-InsR) and total protein of InsR (T-InsR). For protein samples pulled down by IP against total protein of InsR, similar SDS-PAGE coupled with IB using the antibody for GHR was also performed. The IB signals for the two forms of InsR and GHR were quantitated by Image J and presented in the bar graphs below the IB results. In this study, IP with mouse IgG followed by IB for the respective targets was used as the negative control **(B)**. Parallel IB using the protein extract prior to IP was used as the input control while the corresponding blotting for β actin in whole cell lysate was also conducted to serve as the loading control **(C)**.

孵育15min，得到膜蛋白

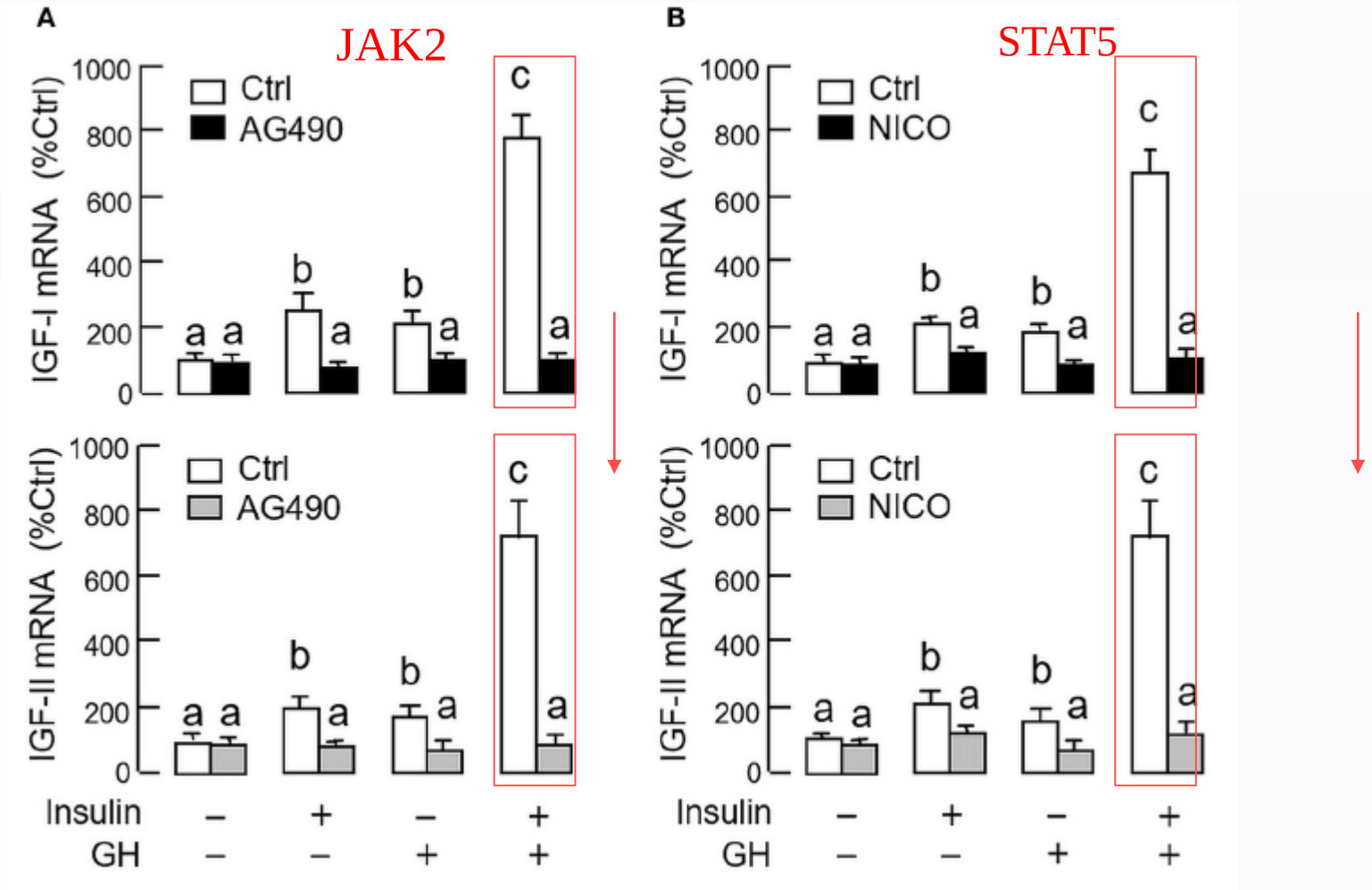



**FIGURE 7** | Functional role of $JAK_2/STAT_5$ pathway in the synergistic effect of GH and insulin on IGF-I and -II expression in carp hepatocytes. **(A)** $JAK_2$ blockade or **(B)** $STAT_5$ inactivation on insulin potentiation of GH-induced IGF-I and -II mRNA expression at the hepatic level. In this study, hepatocytes were challenged for 24 h with insulin alone (10 nM), GH alone (300 ng/ml) or co-treatment with GH (300 ng/ml) and insulin (10 nM) in the presence of the $JAK_2$ inhibitor AG490 (20 μM) or $STAT_5$ inactivator NICO (20 μM). After treatment, total RNA was isolated and used for real-time PCR for IGF-I and -II mRNA. For IGF data presented, the groups denoted by different letters represent a significant difference at $P < 0.05$. 孵育24h

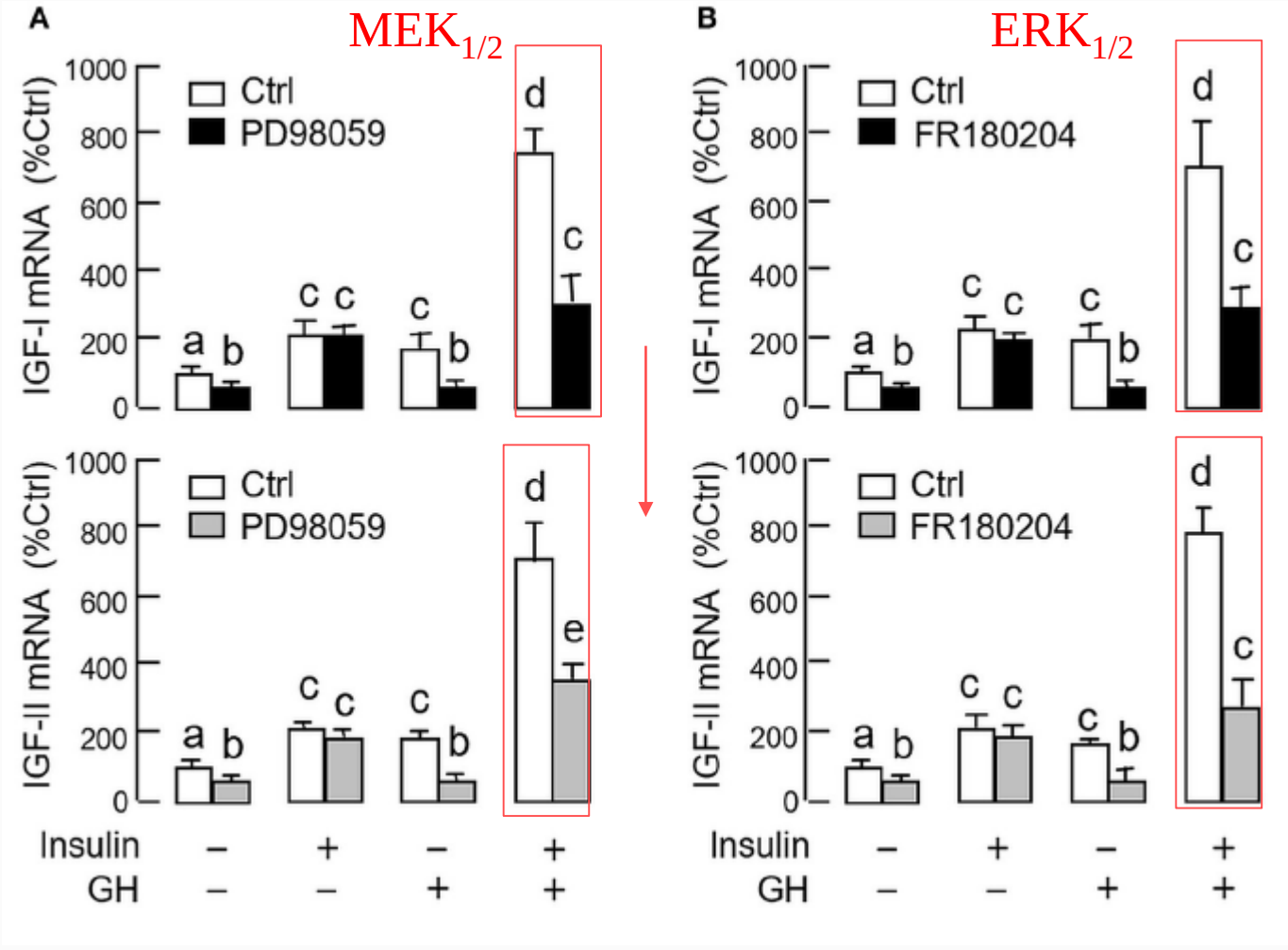



**FIGURE 8 |** Functional role of $MEK_{1/2}$/$ERK_{1/2}$ pathway in the synergistic effect of GH and insulin on IGF-I and -II expression in carp hepatocytes. **(A)** $MEK_{1/2}$ blockade or **(B)** $ERK_{1/2}$ inhibition on insulin potentiation of GH-induced IGF-I and -II mRNA expression at the hepatic level. In this study, hepatocytes were challenged for 24 h with insulin alone (10 nM), GH alone (300 ng/ml) or co-treatment of GH (300 ng/ml) and insulin (10 nM) in the presence of the $MEK_{1/2}$ inhibitor PD98059 (10 μM) or $ERK_{1/2}$ inhibitor FR180204 (2 μM). After that, total RNA was isolated and used for real-time PCR for IGF-I and -II mRNA. For IGF data presented, the groups denoted by different letters represent a significant difference at $P < 0.05$. 孵育24h

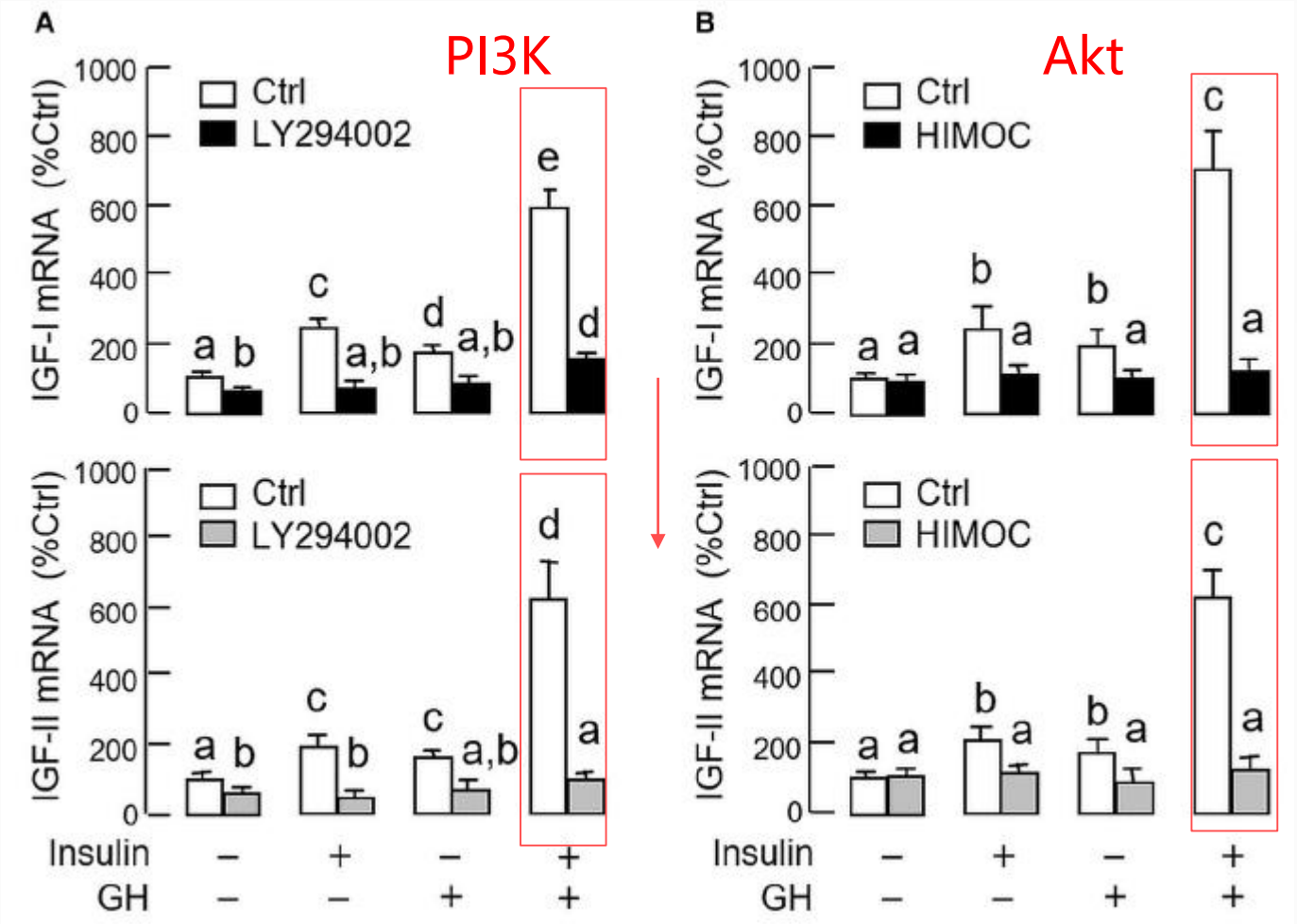



**FIGURE 9 |** Functional role of P38 MAPK and PI3K/Akt pathways in the synergistic effect of GH and insulin on IGF-I and -II expression in carp hepatocytes. Effects of inhibiting **(A)** PI3K, **(B)** Akt, **(C)** mTOR and **(D)** P38 MAPK on insulin potentiation of GH-induced IGF-I and -II mRNA expression at the hepatic level. Hepatocytes were incubated for 24 h with insulin alone (10 nM), GH alone (300 ng/ml) or co-treatment with GH (300 ng/ml) and insulin (10 nM) in the presence of the PI3K inhibitor LY294002 (10 μM), Akt inhibitor HIMOC (10 μM), mTOR inhibitor rapamycin (20 nM) and P38 MAPK inhibitor SB203580 (5 μM), respectively. After that, total RNA was isolated and used for real-time PCR for IGF-I and -II mRNA. For IGF data presented, the groups denoted by different letters represent a significant difference at $P < 0.05$.

孵育24h

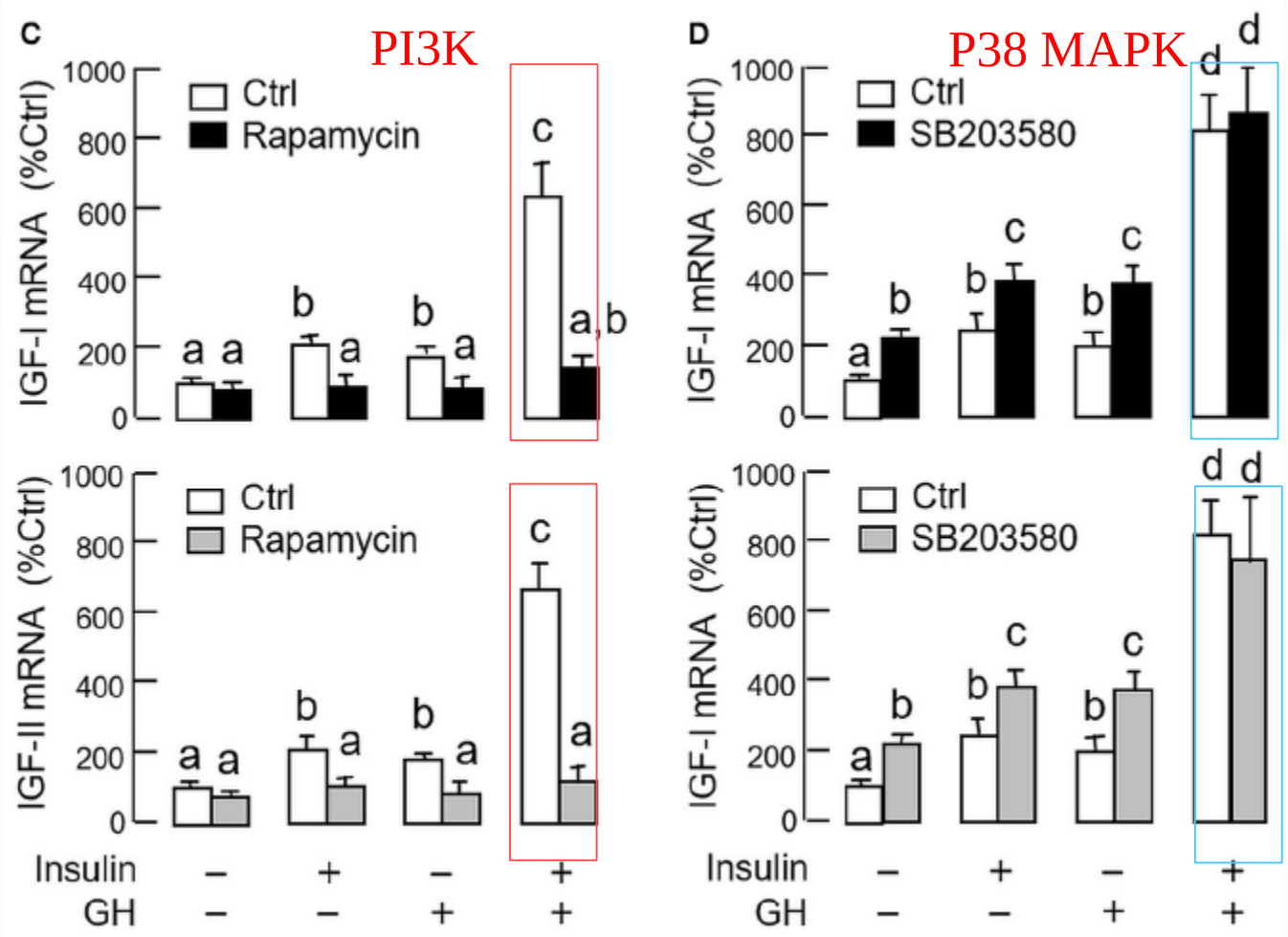



**FIGURE 9 |** Functional role of P38 MAPK and PI3K/Akt pathways in the synergistic effect of GH and insulin on IGF-I and -II expression in carp hepatocytes. Effects of inhibiting **(A)** PI3K, **(B)** Akt, **(C)** mTOR and **(D)** P38 MAPK on insulin potentiation of GH-induced IGF-I and -II mRNA expression at the hepatic level. Hepatocytes were incubated for 24 h with insulin alone (10 nM), GH alone (300 ng/ml) or co-treatment with GH (300 ng/ml) and insulin (10 nM) in the presence of the PI3K inhibitor LY294002 (10 μM), Akt inhibitor HIMOC (10 μM), mTOR inhibitor rapamycin (20 nM) and P38 MAPK inhibitor SB203580 (5 μM), respectively. After that, total RNA was isolated and used for real-time PCR for IGF-I and -II mRNA. For IGF data presented, the groups denoted by different letters represent a significant difference at $P < 0.05$. 孵育24h

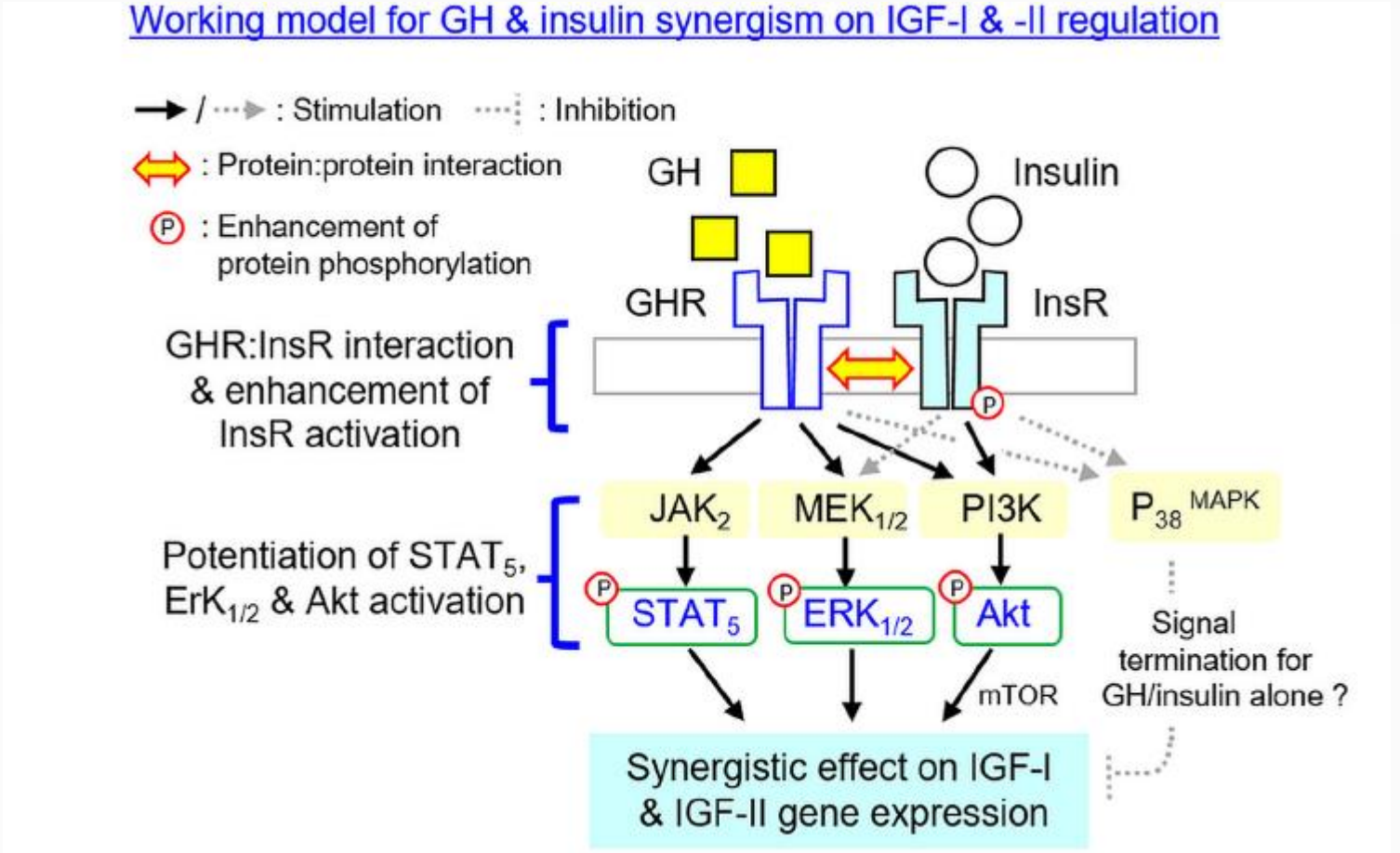



**FIGURE 10 |** Working model proposed for the synergistic action of GH and insulin on IGF-I and -II expression in the carp liver. In carp hepatocytes, IGF-I and -II mRNA expression can be up-regulated by GH via the $JAK_2/STAT_5$, $MEK_{1/2}/ERK_{1/2}$, and PI3K/Akt/mTOR cascades. Insulin also has similar effects on IGF-I and -II gene expression but these actions are mediated via PI3K/Akt/mTOR but not $JAK_2/STAT_5$ and $MEK_{1/2}/ERK_{1/2}$ pathways. At the hepatic level, IGF-I and -II responses induced by GH can be markedly enhanced with insulin co-treatment. This potentiating effect can be observed with protein:protein interaction of GHR and InsR at the membrane level together with notable enhancement in InsR phosphorylation. Meanwhile, the levels of $STAT_5$, $ERK_{1/2}$, and Akt phosphorylation can also be potentiated by GH and insulin co-treatment. The aggravation in $JAK_2/STAT_5$, $MEK_{1/2}/ERK_{1/2}$ and PI3K/Akt signaling caused by simultaneous activation of GHR and InsR probably can contribute to the synergist effect on IGF-I and -II expression. Of note, $MEK_{1/2}$ and $ERK_{1/2}$ activation can also be induced by insulin but the pathway is not involved in insulin-induced IGF-I and -II expression. However, the functional role of $MEK_{1/2}/ERK_{1/2}$ activation by insulin in the potentiating effects caused by GH and insulin co-treatment should not be excluded. In carp model, P38 MAPK of MAPK cascades is not involved in the synergistic action of GH and insulin. P38 MAPK may play a role in signal termination for the IGF responses induced by GH or insulin respectively, and interestingly, its inhibitory effect can be nullified by co-treatment with GH and insulin but the mechanisms involved have yet to be elucidated.

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1.GH可通过JAK2/STAT5、 $MEK_{1/2}$/$ERK_{1/2}$和PI3K/AKT/mTOR通路来上调IGF-I和IGF-II的表达，而insulin则通过JAK2/STAT5、 $MEK_{1/2}$/$ERK_{1/2}$来上调IGF-I和IGF-II的表达。

2. GH和insulin协同作用可通过受体蛋白相互作用和增强后受体信号来上调IGF-I和-II的表达。

# Thank You