GDF9 and BMP15: Species Difference and Synergistic Interactions

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Abstract

GDF9 and BMP15 are two oocyte-secreted proteins which have been shown to be essential for normal mammalian fertility. There are a number of factors which impact their efficiency, including species difference of the proteins, GDF9/BMP15 interactions and the presence of post-translational modifications. However, factors such as species difference and post-translational modifications have yet to be investigated in terms of their effects on GDF9/BMP15 interactions.

The aims of this study were to produce well purified human and mouse GDF9 and human BMP15 to test the effects of these factors. We found clear species differences between mouse and human GDF9 in thymidine incorporation in mouse and bovine granulosa cells. However, not only did we find a species difference due to the species of the protein, but also a difference due to the species of the cells on which the proteins were acting. GDF9 and BMP15 were found to interact synergistically on mouse granulosa cells, but not on bovine cells. Human GDF9 was found to be dependent on the presence of BMP15 for bioactivity in both species of cell however, the introduction of mouse-like residues into the human GDF9 sequence was able to produce a protein capable of functioning independent of BMP15 and with even higher bioactivity than wild-type mouse GDF9.

Post-translational modifications were also found to have significant effects on synergistic GDF9/BMP15 synergistic interactions. Both GDF9 and BMP15 have previously been shown to be phosphorylated. This appears to be more important to the correct functioning of GDF9. Loss of GDF9 phosphorylation was found not only to decrease its bioactivity but also to decrease its synergistic interactions with BMP15. Phosphorylation was not found to affect BMP15 however; the loss of o-linked glycosylation decreased its ability to synergise with GDF9. To fully assess the implications and applications of this work, there is still a great deal of work to be done however, it is clear that for any embryological studies, the species differences of the proteins and cells need to be carefully considered.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Dedication

To my wonderful family and friends.

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Abstracts Arising from This Thesis

TGF-β DownUnder Conference – 2011

Studies on the function of the pro-regions of BMP15 and GDF9

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Bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) are two oocyte secreted TGF-β superfamily proteins which have been shown to be essential for normal mammalian fertility. Similar to other TGF-β proteins, the mature regions of BMP15 and GDF9 maintain a close association with their corresponding pro-regions after processing, however it is not known if the BMP15/GDF9 pro-region plays a role in the bioactivity of the corresponding mature region. The aim of this study was to produce purified forms of both the BMP15 and GDF9 pro-mature complexes and isolated mature regions in order to gauge the function of the pro-region. Human BMP15 and GDF9 were produced by transfecting human embryonic kidney 293T cells with plasmids containing BMP15 or GDF9 expression cassettes with poly-histidine and StrepII affinity tags engineered into the N-terminus of the pro-region. The pro-mature complexes of both proteins were purified from conditioned media using two affinity purification steps using the poly-histidine and StrepII tags. The mature regions were isolated from the purified pro-mature complexes using an additional step of reverse phase high performance liquid chromatography. The BMP15 and GDF9 mature regions and pro-mature complexes were then compared in mouse granulosa cell

proliferation assays. The resulting data showed that the mature regions of human BMP5 and GDF9 were significantly more bioactive than the corresponding pro-mature complexes. This suggests that the pro-regions of both human BMP15 and GDF9 are inhibitory to the actions of the corresponding mature regions in mouse granulosa cells.

World Congress on Reproductive Biology Conference – 2011

BMP15/GDF9 Synergistic Interactions

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BMP15 and GDF9 are two TGF-β superfamily proteins which are essential for normal mammalian fertility. Both proteins share a similar pre-propeptide structure and exist after proteolytic processing as a pro-mature complex. Both BMP15 and GDF9 have been shown, among other functions, to stimulate granulosa cell proliferation. Previous studies using unpurified preparations of recombinant mouse or ovine BMP15 and GDF9 have indicated that these proteins display synergistic interactions. Further, recently it has been suggested that there are species differences with regard to GDF9/BMP15 synergistic responses. The purpose of this study was to investigate the synergistic interactions of purified recombinant human BMP15 and GDF9. The proteins were produced by transfecting human embryonic kidney 293T cells with plasmids containing BMP15 or GDF9 expression cassettes with a poly-histidine tag engineered into the N-terminus of the pro-region. The purified mature regions of these proteins were obtained via a single step of His tag affinity chromatography followed by two steps of reverse phase high performance liquid chromatography. The effects of the

human BMP15 and GDF9 mature regions, both individually and together, were measured using [^{3}H]-thymidine incorporation in primary mouse granulosa cells. Here we present our recent results characterising the synergistic interactions of our purified human GDF9 and BMP15 proteins in comparison with those observed utilizing a purified commercially available mouse GDF9.

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Abbreviations

GDF9	Growth and differentiation factor 9 (h and m denote human or mouse GDF9)
BMP15	Bone morphogenetic protein 15
GDF9 Mut 1	Human GDF9 with a single mouse-like $Gly^{391}Arg$ mutation
GDF9 Mut 2	Human GDF9 with multiple mouse-like mutations
GDF9 S7A	GDF9 Mut 2 with phosphorylation sites eliminated
BMP15 T277A	BMP15 with o-linked glycosylation sites eliminated
BMP15 S/T	BMP15 with o-linked glycosylation and phosphorylation sites eliminated.
aa	Amino acid
ACN	Aceto-nitrile
ALK5/ALK6	Activin-like kinase 5/6
ANOVA	Analysis of Variance
Arg	Arginine
BMPRII	Bone morphogenetic protein receptor type 2
CC	Cumulus cell
CHO cells	Chinese hamster ovarian cells
CM	Conditioned media
Cys	Cysteine
DMEM	Dulbecco's modified eagle medium
DTT	Dithiothreitol

EGF	Epidermal growth factor
ERK1/2	Extracellular signal-regulated kinases 1/2
F	Furin-like processing site
FCS	Foetal calf serum
GC	Granulosa cell
Gly	Glycine
Has2	Hyaluronan synthase 2
HEK293T cells	Human Embryonic kidney 293T cells
hGL	Human granulosa luteal cells
IGF-1	Insulin-like growth factor 1
IMAC	Immobilised metal affinity chromatography
IVF	*In vitro* fertilisation
IVM	*In vitro* maturation
kD	Kilo daltons
KGN	Human granulosa-like tumour cell line
MAPK	Mitogen-activated protein kinase
mRNA	Messenger ribo nucleic acid
nFκ-β	Nuclear factor κ-β
PBS	Phosphate buffered saline
pEF-IRES	Elongation factor – internal ribosomal entry site plasmid
PMSG	Pregnant mare serum gonadotropin
Ptgs2	Prostaglandin-endoperoxide synthase 2
rpHPLC	Reversed phase high performance liquid chromatography
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ser	Serine

SS	Signal sequence
TFA	Trifluoro-acetic acid
TGF-β	Transforming growth factor β
Thr	Threonine