# The effect of GLP-1 and Obestatin in the generation of insulin producing cells from Wharton's jelly mesenchymal stem cells


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## Introduction

- The number of patients suffering from Diabetes Mellitus (DM) is growing in an alarming rate[1]
- In fact, insulin secreting β-cells are damaged to different extent in both type 1 and 2 diabetic patients[2].
- The major goal of future diabetes therapy is to promote β-cell regeneration through stem cell therapy[3].
- Recently, the umbilical cord (UC) has been proved to be a good source of mesenchymal stem cells (MSCs) especially from Wharton's Jelly (WJ); the connective tissue surrounding the umbilical vessels[4].
- Generation of Insulin producing cells (IPCs) from WJ-MSCs is still a challenge[5].
- GLP-1, a gut hormone, has been implicated in the differentiation of MSCs into IPCs[5]
- Obestatin, another gut hormone, has been recently shown to improve generation of functional β-cells from pancreatic mesenchymal stem cells[6]

## Objective

***In this study we aimed to:***

- ***Isolate, propagate and characterize MSCs from WJ as non-invasive and readily available source of stem cells***
- ***Examine the effect of gut hormones including GLP-1 and obestatin in generation of IPCs in-vitro from WJ-MSCs in comparison to exendin-4.***

## Materials and Methods

**To fulfill the aim of this study we did the following experiments:**





**1) The UCs were obtained from Obstetrics/Gynecology Department, Ain Shams University Hospitals.**

**2) Isolation of WJ-MSCs by explant method [7]**

**3) Characterization of isolated cells from WJ[8]:**

a) Adherent Culture in 10% FBS-LGDMEM
b) Immunophenotyping by Flowcytometry
c) Adipogenic differentiation

**4) Effect of gut hormones on stem cell markers of WJ-MSCs: WJ-MSCs under proliferation conditions (10%FBS-DMEM) were incubated for10 days with either:**

a) 10 nM Exendin-4
b) 10 nM GLP-1
c) 100 nM Obestatin

**5) Differentiation of WJ-MSCs into IPCs**

**Short Protocol[9]**
2 days: LG-DMEM+10 mM nicotinamide +1 mM β-mercaptoethanol in
1 day: HG-DMEM+10 mM nicotinamide + 1 mM β-mercaptoethanol
7 days: HG-DMEM + either:
a) 10 nM exendin-4
b) 10 nmol/L GLP-1
c) 100 nmol/L Obestatin

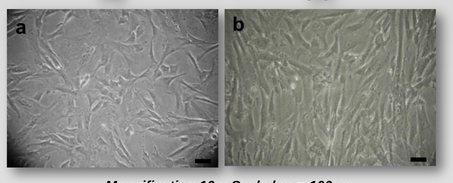
**Long protocol[10]**
14 days: HG-DMEM+5% FBS (Step I)
7days : HG-DMEM+5% FBS+10 mmol/L nicotinamide (Step II)
7 days: HG-DMEM+5% FBS+10 mmol/L nicotinamide + either (Step III):
a) 10 nmol/L exendin-4
b) 10 nmol/L GLP-1
c) 100 nmol/L Obestatin

**6) Assessment of Pancreatic lineage differentiation:**
a) mRNA gene expression (qRT-PCR)
b) Glucose stimulated Insulin secretion (GSIS)

## Results

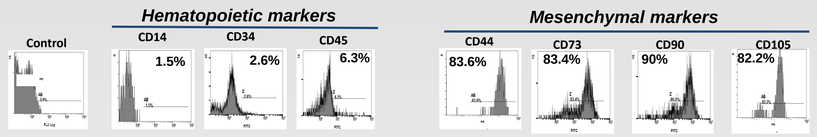
***WJ is a sources of MSCs***

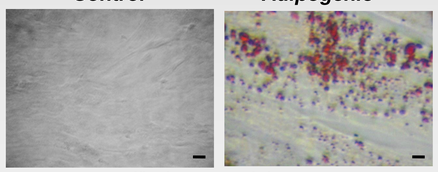
***Freshly isolated WJ-MSCs showing homogenous fibroblast-like cells at (a) P0 and (b) P1***




*Magnification 10x; Scale bar = 100µm*

***WJ-MSCs lack hematopoietic CDs and express mesenchymal CDs***

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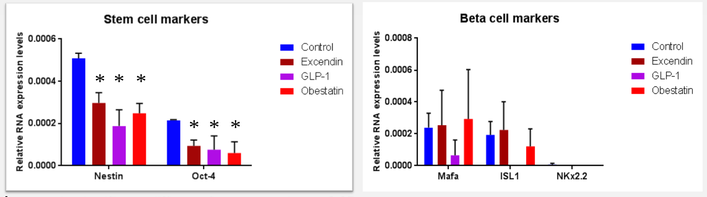
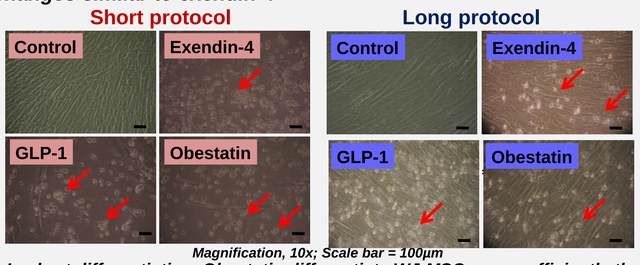
***WJ-MSCs exhibited adipogenic differentiation showed by oil droplets staining by Oil-Red***

***Both GLP-1 and Obestatin decreased stem cell markers, nestin and Oct-4 expression in WJ-MSC under proliferation conditions, but failed to express β-cell markers***




**: mean is significant different from control at p<0.05*

***Both short and long differentiation by GLP-1 and Obestatin induced morphological changes similar to exendin-4***

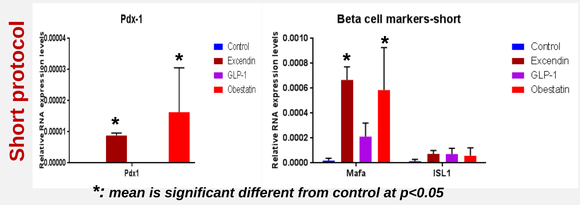



*Upon differentiation, WJ-MSCs aggregate to form clusters in contrast to control cells which retain fibroblast-like morphology*

*Magnification, 10x; Scale bar = 100µm*

***In short differentiation, Obestatin differentiate WJ-MSCs more efficiently than GLP-1 and similar to exendin-4***

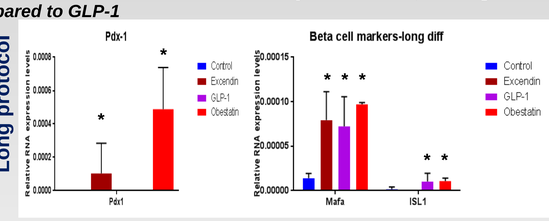
***Obestatin induced more expression of Pdx-1 and Mafa as compared to GLP-1 and in similar pattern to exendin-4***



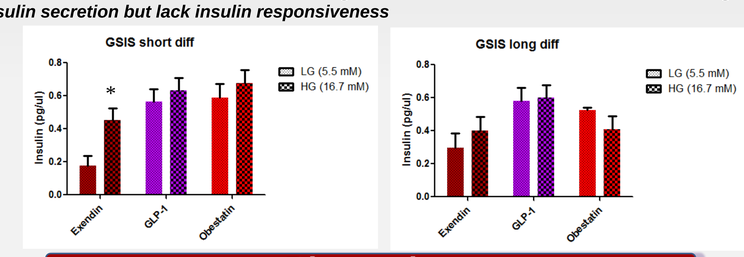

**: mean is significant different from control at p<0.05*

***In long differentiation, Obestatin differentiate WJ-MSCs similarly to exendin-4, with higher expression of Pdx-1, as compared to GLP-1***

***Obestatin induced more expression of Pdx-1 as compared to GLP-1 and exendin-4***




***In vitro GSIS assay: Insulin release in response to low (5.5 mM) and high (16.7 mM) glucose concentrations of differentiated cells. IPCs generated from GLP-1 or obestatin showed higher insulin secretion but lack insulin responsiveness***




## Discussion

- WJ represents a source of MSCs which yields homogenous population that can be easily isolated and possesses all MSCs characteristics.
- Under proliferation conditions, both gut hormones, GLP-1 and Obestatin, together with exendin-4, induced exit of WJ-MSCs from stemness state.
- WJ-MSCs can be differentiated to IPCs using exendin-4, GLP-1 and Obestatin using different protocols.
- Under short and long differentiation protocols, obestatin, as well as exendin-4, induced expression of β-cell markers, while GLP-1 failed to show similar effect.
- As for GSIS, IPCs generated with GLP-1 and obestatin showed higher secretion of insulin , while those generated by exendin-4 showed more glucose responsiveness than both.

## Conclusions and Recommendations

- ***Gut hormones including GLP-1 and Obestatin can generate IPCs from WJ-MSCs.***
- ***Obstatin is an effective differentiating factor comparable to exendin-4 and may be better than GLP-1.***
- ***Obestatin should be considered a novel differentiating marker for optimization protocols.***
- ***Mechanism of Obestatin effect on generation of IPCs from MSCs should be elucidated.***

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