

Effects of Leukemia Inhibitory Factor on gp130 Expression and Rate of Metaphase II Development during *in vitro* Maturation of Mouse oocyte

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ABSTRACT

Background: Leukemia inhibitory factor (LIF) is a 45-56 kDa glycoprotein that has an important role in proliferation and embryo implantation. Its effect on oocyte maturation and how to exert the function remained to be elucidated. **Methods:** Immature mice superovulated with human menopausal gonadotropin and germinal vesicle (GV) oocytes were obtained from ovary 48 hours after. GV oocytes were cultured in tissue culture medium 199 with 0, 100, 500 and 1,000 U/ml LIF. Cumulus expansion and *in vitro* maturation (IVM) rate were assessed after culture. For reverse transcriptase PCR, total RNA from GV and metaphase II (MII) oocytes were extracted by Trizol reagent. The quantity and quality of RNA were determined by spectrophotometry and electrophoresis, respectively. Reverse transcription was performed by Super Script III reverse transcriptase with 1 µg of total RNA followed by DNase I treatment and heat inactivation. Expression of gp130 was determined by RT-PCR. **Results:** Our results showed that cumulus expansion was improved with 1,000 U/ml in culture medium compared to others. GV breakdown and MII rate in groups with LIF were higher than control group and were dose dependant. In 1,000 U/ml, LIF rate of MII was significantly higher than control group ($P < 0.05$). Our results also showed that gp130 is expressed neither in GV nor in MII oocytes during IVM of mouse oocytes. **Conclusion:** gp130 is expressed in human oocyte but not in mouse. Our results suggest that in mouse, LIF could affect the oocyte via another receptor or via cumulus cells; however, further studies are warranted. *Iran. Biomed. J.* 14 (3): 103-107, 2010

Keywords: Leukemia inhibitory factor (LIF), Oocytes, *In vitro* maturation (IVM)

INTRODUCTION

The *in vitro*-matured oocytes are known to have a lower developmental competence after *in vitro* fertilization to *in vivo*-derived oocytes. Suboptimal culture medium is the most important factor for obtaining high quality matured oocytes. Recent studies have effort to obtain an optimized culture media to improve *in vitro* maturation (IVM) rate [1, 2].

Leukemia inhibitory factor (LIF), a pleiotropic cytokine, is expressed and actively involved in a wide variety of cell and tissue types. LIF is essential for implantation of mouse embryos, also promotes

the transition of primordial to primary follicle in cultured ovaries and may be an important element of regulatory pathways controlling recruitment of follicles from the primordial follicle pool [3-5]. In other hand, recent studies suggest that endometrial LIF contributes to human infertility [6]. However, its effect on oocyte and embryo development is controversy and more studies are required.

Previous studies have found that LIF might affect on embryo development and blastocysts implantation; however, little attention has been paid to understand its effects on oocyte maturation [7]. LIF is present in human follicular fluid and its levels are regulated according to the stage of antral follicle

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development. LIF levels in follicular fluid are also responsive to human chorionic gonadotropin [3, 4]. To further understanding its effect during IVM of mouse oocyte, this study was employed.

LIF exerts its effects by binding to cell surface receptors. Equilibrium analysis of the binding of radio-iodinated LIF led to the classification of two receptor types [8, 9]. The LIF receptor complex is a heterodimer composed of LIF receptor β and gp130. It has been proved that gp130 is expressed in human but there is no report about its expression in mouse oocytes [10]. For further understanding the molecular mechanism of LIF on oocyte maturation, expression of gp130 as a LIF receptor during IVM of mouse oocyte has been investigated in the current study.

MATERIALS AND METHODS

Animals. Oocytes were obtained from immature female BALB/c mice with age of 4 weeks that were kept under controlled light and temperature conditions with free access to water and food. They had 12 hour light and 12 hour dark condition. Animal experiments were approved by the Ethnical Committee of Tabriz Medical University and performed with accordance with the University guidelines.

Collection of germinal vesicle (GV) oocytes. For obtaining GV stage oocytes, mice were primed with 7.5 IU of human menopausal gonadotropins. After 45-50 hours, the animals were sacrificed by cervical dislocation, and the ovaries placed in TCM-199 with 10% fetal bovine serum and 100 IU penicillin-streptomycin. Cumulus oocytes complexes were retrieved directly from the follicles under a stereomicroscope by two 27-gauge needles.

In vitro maturation. IVM medium consisted of TCM-199 supplemented with 10% fetal bovine serum, 0.23 mM sodium pyruvate, 75 mIU follicular stimulating factor and different concentration of LIF (100, 500 and 1,000 U/ml and none as control). Cumulus-oocytes complexes (n = 15-20) were transferred to 50 μ l droplet covered with mineral oil and cultured in 37°C and 5% CO₂.

Cumulus expansion. After IVM, cumulus expansion in different groups was assessed blindly with 2 different persons. Cumulus expansion characterized in 2 groups: 1 plus (+) and 2 plus (++) according to the cumulus cell layers.

GV breakdown (GVBD) and metaphase II (MII) assessment. Cumulus cells were denuded with %0.1 hyaluronidase and rate of GVBD and MII was recorded in different groups.

mRNA extraction from GV and MII oocytes. For detection of gp130 expression, RT-PCR was performed. Total RNA was extracted from GV, MII oocytes and 2-cell embryos and mouse uterus (control groups) by Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The quantity and quality of RNA were determined by spectrophotometry (ND-1000; Nanodrop, Wilmington, DE, USA) and through electrophoresis (Bio Rad, Canada), respectively.

cDNA synthesis. Reverse transcription was performed by Super Script III reverse transcriptase (Invitrogen, Carlsbad, CA) with 1 μ g of total RNA followed by DNaseI (Invitrogen, Carlsbad, CA) treatment and heat inactivation.

Assessment of gp 130 expression in GV and MII oocytes. Semi-quantitative PCR was performed using Taq DNA polymerase (Roche Diagnostic, Germany) in a GeneAmp PCR system 9600 (PerkinElmer Life and Analytical Sciences, Wellesley, MA). After initial denaturation (5 min at 94°C), cDNA was subjected to 30 cycles of PCR. Primer set for the mouse gp130 5'-CAGCAGCAGGTTTCAGATCAC-3 and reverse 5'-GGACAAAGTCACACCTGGGC-3. For the normalization, expression of β -actin was examined and the primer set was forward 5'-TTC TAC AAT GAG CTG CGT GTG G-3' and reverse 5'-GTG TTG AAG GTC TCA AAC ATG AT-3'. PCR annealing temperature was 59°C for mouse gp130 and 59°C for β -actin. PCR products were evaluated in a 2% agarose gel.

Statistical analysis. Comparisons among groups of oocytes were performed using Chi-square and ANOVA with the Tukey-Kramer multiple comparison test as a post test. All values are expressed as the Mean \pm SD. A probability of $P < 0.05$ was considered statistically significant. Oocyte culture was repeated in four and PCR Results are expressed as three independent experiments.

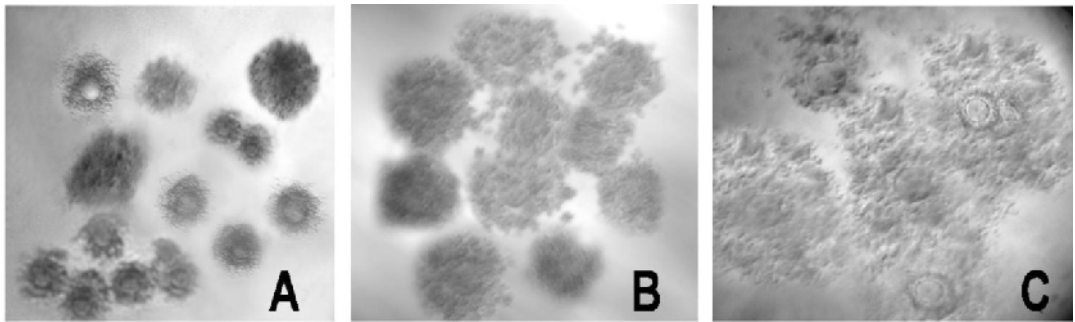


Fig 1. Cumulus expansion. After culturing of GV oocytes, Cumulus expansion was assessed as + and ++. Cumulus expansion in control Group with more non-expanded oocytes (A), cumulus expansion in group with 500 U/ml LIF with more + expanded oocytes (B) and cumulus expansion in 1,000 U/ml LIF with more ++ expanded oocytes (C).

RESULTS

Cumulus expansion. Our results showed that LIF in culture medium improved cumulus expansion in dose dependent. ++ cumulus expansion was higher than in 1,000 IU/ml compare to other groups. + cumulus expansion was higher significantly in 500 IU/ml LIF compare to the control group ($P<0.05$). Also in control group unexpanded oocytes was higher than other groups (Fig. 1).

GVBD and MII development. For nuclear development, rate of GVBD and MII oocytes have been recorded. Our results revealed that culture of GV oocytes in medium with LIF improved nuclear maturation and rate of GVBD and MII was higher compared to control. Rate of MII in 1,000 IU/ml LIF had highest improvement and was significant ($P<0.05$, Fig. 2A and 2B).

Expression of gp 130 in GV and MII oocytes. To determine whether gp130 is expressed in GV and MII oocytes, RT-PCR was performed. Expression of gp130 was observed in the control samples (mouse uterus and 2-cell embryo) but there was no expression in GV and MII oocytes during IVM (Fig. 3).

DISCUSSION

We have demonstrated that LIF is capable to induce mouse cumulus expansion and MII development rate. Cumulus expansion depends on the matrix on which the granulosa cells moves. Three major factors are involved in the Cumulus expansion. These three components are hyaluronic acid [11, 12] and at least two hyaluronic acid-binding proteins i.e. tumor necrosis factor-stimulated

gene-6 and the serum derived inter-a-inhibitor). Signaling by LIF as well as several IL-6 cytokine family members involve the Jak-Stat cascade and the Ras-MAPK pathway [13] followed by activation of mentioned proteins. Thus, LIF *in vitro*-induced cumulus expansion could be mediated by MAPK activity. One possibility is that LIF may act to

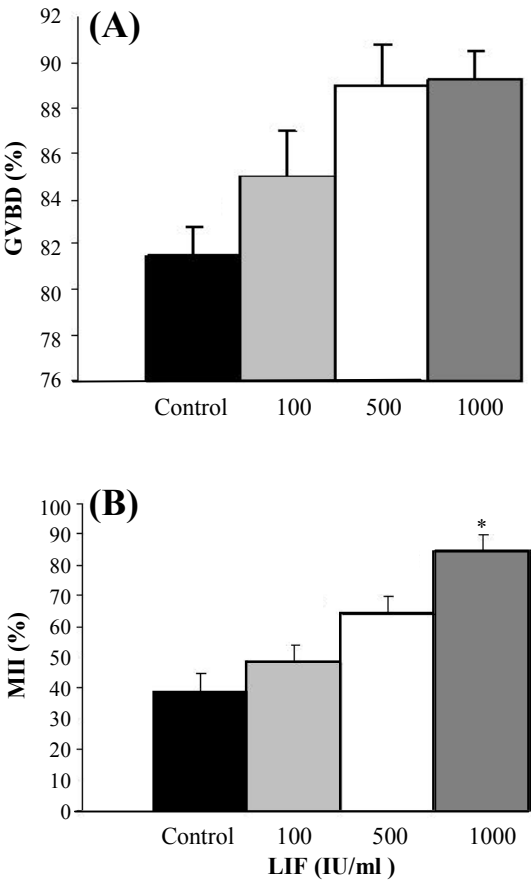


Fig 2. Nuclear maturation rate in different doses of LIF IU/ml. (A) GVBD rate shows that with LIF, nuclear maturation improved and in (B) MII oocyte development increased in dose-dependent manner; * shows $P<0.05$ was considered statistically significant.

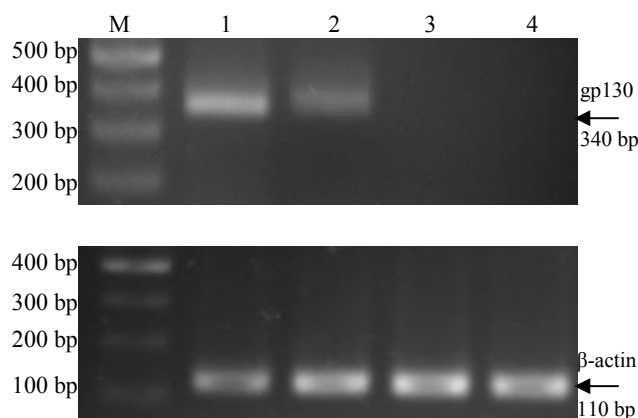


Fig 3. Lack of expression of gp130 gene in Germinal vesicle (GV) and MII stage oocyte. Upper one is expression of gp130 in uterus (lane 1), 2-cell embryo (lane 2), GV oocyte (lane 3) and MII oocyte (lane 4). Lower one is β -actin expression in groups as mentioned. M, molecular weight.

increase expression of growth factors that in turn affect granulosa or theca cell proliferation [11].

Oocyte maturation includes nuclear and cytoplasmic maturation. Supplementation of culture medium with LIF could improve nuclear maturation with increasing of GVBD and MII rate [14]. Cumulus expansion increase gap junction and removes inhibition of granulosa cells on meiosis resumption [15]. In our study in concurrent with improvement of cumulus expansion, rate of GVBD and MII increased specially with 1,000 U/ml LIF. Ptak and his colleagues [14] reported that LIF enhances sheep fertilization *in vitro* via an influence on the oocyte. In this study, 1,000 U/ml concentration of LIF improved oocyte maturation compared to control [14]. In another study, De Matos *et al.* [11] found that 1,000 ng/ml LIF induces cumulus expansion in immature human and mouse oocytes. These results are concurrent with our study.

It is suggested that in a paracrine manner, LIF might signal from the pre-granulosa cells of primordial follicles to either the arrested oocyte or to surrounding stromal tissue. Such signaling, alone or in combination with other factors, could promote the primordial to primary follicle transition. LIF expression becomes to be higher in later antral follicles stage in the oocyte. LIF may also have a different signaling role in these later follicles than in primordial follicles [16].

Mechanisms underlying LIF effect on oocyte maturation is unknown [14]. LIF as a ligand binds to its receptors, LIF and gp130. We showed that gp130 is not expressed in mouse oocyte membrane during IVM; however, cumulus cells around oocytes

express LIF receptors mentioned in another studies. Probably, LIF could improve cumulus expansion and maturation rate via cumulus cells indirectly [17, 18].

In conclusion, our results demonstrated that LIF could improve cumulus expansion and oocyte maturation in a dose-dependent manner. Our result revealed that optimal concentration of LIF for oocyte maturation in mouse was 1,000 IU/ml. Our results also showed that LIF receptor (gp130) is not expressed in oocyte membrane and more probably, LIF effects indirectly throughout cumulus cells. However, to understand the mechanism of LIF on oocyte maturation, further studies are required.

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