MELATONIN AS MODULATOR OF ANGIOPOIETINS EXPRESSION IN HUVEC AND MCF-7 CELLS

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INTRODUCTION

- Melatonin, the main secretory product of the pineal gland, exerts oncostatic effects on breast cancer by interfering with the estrogen-signaling pathways. Melatonin reduces estrogen biosynthesis in human breast cancer cells, as well as surrounding fibroblasts and peritumoral endothelial cells by regulating cytokines secretion that influence tumor microenvironment. This hormone also exerts antiangiogenic activity in tumoral tissues.
- Angiogenesis is the formation of new blood vessel from existing endothelium. This process is involved in tumor's progression and development. The role of angiogenic growth factors as molecular targets in tumor pathology has become relevant because since the inhibition of angiogenesis has been included as another cancer treatment strategy.
- Angiopoietins are cytokines that regulate angiogenesis and the Angiopoietin/Tie2 system is a vascular specific ligand/receptor system that play a critical role in endothelial cell differentiation and blood vessel morphogenesis by regulating angiogenic-remodeling processes such as vessel stabilization/destabilization and pericyte recruitment or loss.
- **Since it is known that melatonin has antiangiogenic actions the aim of the present study was to investigate the regulatory effects of melatonin on angiopoietins 1 and 2 expression and *Tie*2 receptor in human umbilical vein endothelial cells (HUVEC) and human breast cancer cells (MCF-7).**

MATERIALS AND METHODS

MCF-7 and HUVEC cells cultures and cocultures

MCF-7 culture

MCF-7 ($8X10^5$ cells/well) DMEM + 10% FBS → 24h → DMEM + 0,5% DCC → 24h → DMEM + 0,5% DCC + melatonin M^{-3} and M^{-9} → 4h → Cells were collected for extraction of total RNA.

HUVEC culture

HUVEC ($4x10^5$ cells/well) VCBM+2% FBS → 24h → VCBM + 0,5% DCC → 24h → VBCM +0,5% DCC+ melatonin M^{-3} and M^{-9} → 4h → Cells were collected for extraction of total RNA.

MCF-7 and HUVEC coculture

MCF-7 ($30x10^3$cells/well) DMEM+ 10% FBS → 24h → DMEM + 0.5%DCC → 24h → DMEM + 0.5% DCC + melatonin M^{-3} and M^{-9} → 4h → Cells were collected for extraction of total RNA.

HUVEC ($4x10^5$ cells/insert) VCBM+2% FBS → 24h → VCBM + 0.5% DCC → 24h → VBCM +0.5% DCC+ melatonin M^{-3} and M^{-9}

HUVEC and MCF-7 coculture

HUVEC ($5x10^5$ cells/well) VCBM+2% FBS → 24h → VCBM + 0.5% DCC → 24h → VBCM + 0.5% DCC+ melatonin M^{-3} and M^{-9} → 4h → Cells were collected for extraction of total RNA.

MCF-7 ($4x10^5$ cells/insert) DMEM + 10% FBS → 24h → DMEM + 0.5% DCC → 24h → DMEM + 0.5% DCC + melatonin M^{-3} and M^{-9}

GENE EXPRESSION STUDIES

Detection of gene expression by real time PCR

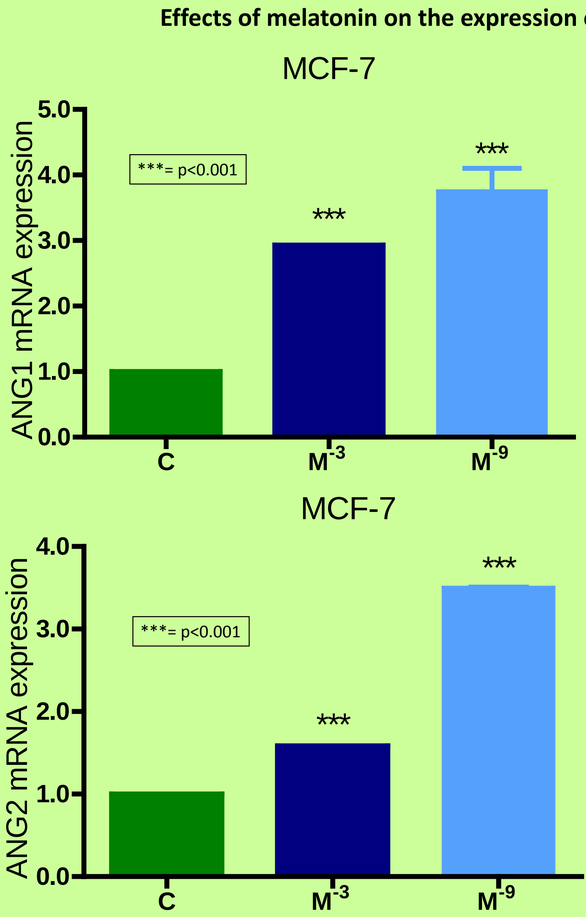
Expression of the mRNA from different gens was carried out by real time polymerase chain reaction (QPCR) in HUVEC and MCF-7 cells. The total cellular RNA was purified using NucleoSpin II (Macherey-Nagel) commercial kit and its integrity was assessed by electrophoresis in ethidium bromide-stained 1.2% agarose-Tris-borate EDTA gels. The absorbance ratio A260/A280 nm was >1.8. For cDNA synthesis, 1 μg of total RNA was denaturated at 65C for 10 min and reverse-transcribed 50 min at 45C with the cDNA synthesis Kit in a final volume of 20 μl in the presence of 500 ng of oligo (dT)12–18 primer. Real time PCRs were performed using Brilliant SYBR Green PCR Master Mix following the manufacturer´s instructions. The sets of human oligonucleotides used as primers were designed with the Beacon designer program. S14 mRNA gene expression was used as reference gene. PCRs were performed for 45 cycles for quantitative analysis using 60º as annealing during 45 s, the extension being carried out at 72C for 30 s and the denaturation at 95C for 30 s. Melting curves were performed by using dissociation curve to verify that only a single product was amplified.

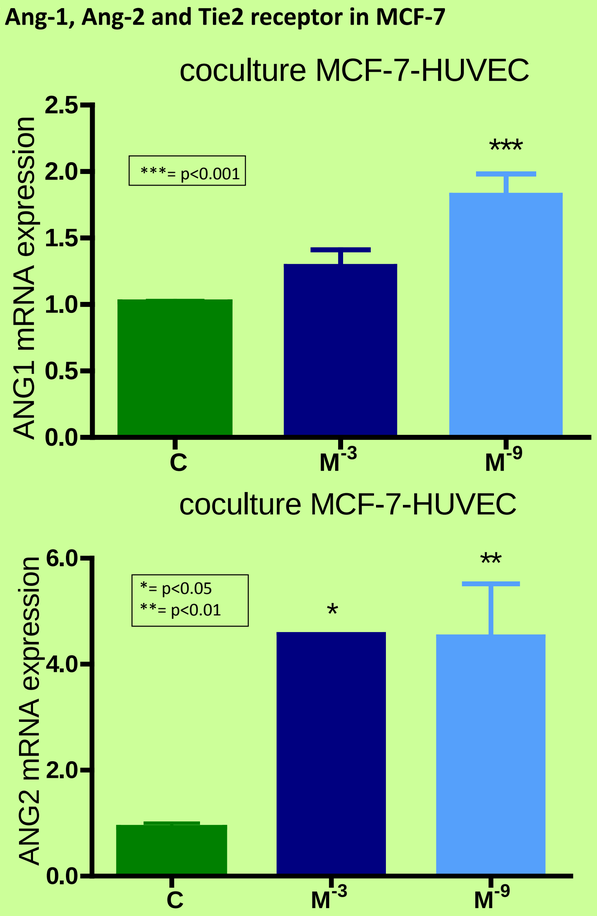
Statistical analysis

The data of mRNA gene expression are expressed as the mean ± standard errors of the mean (SEM). Statistical differences between groups were processed by one way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. Results were considered as statistically significant at $p<0.05$.

RESULTS

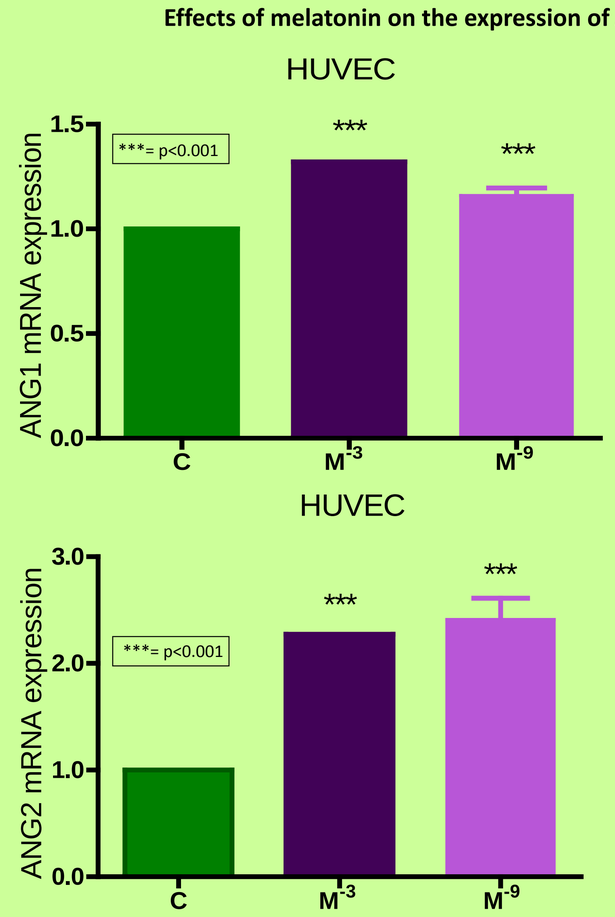
Effects of melatonin on the expression of Ang-1, Ang-2 and Tie2 receptor in MCF-7

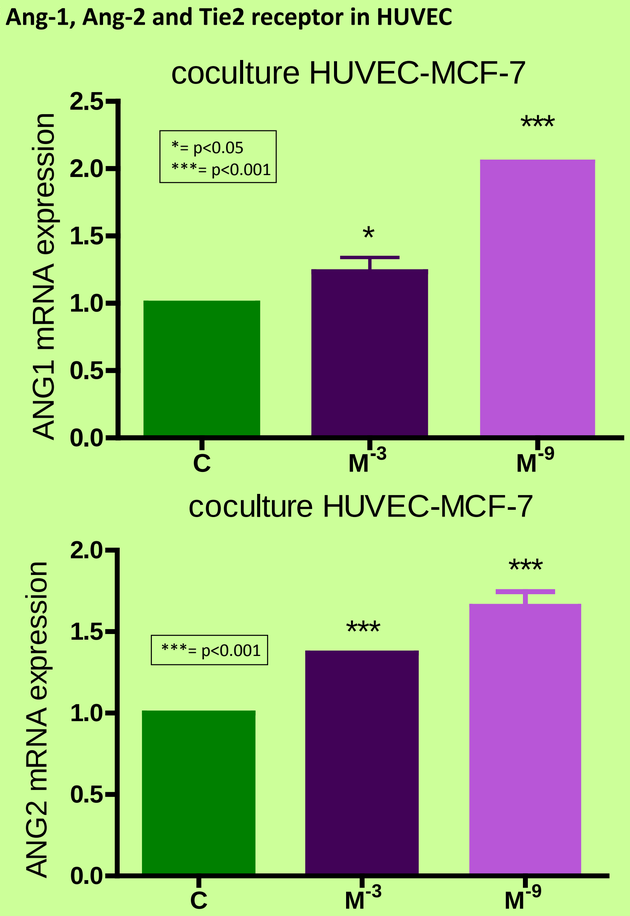


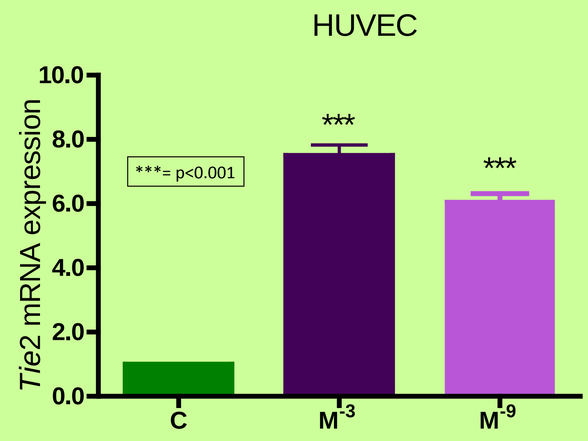


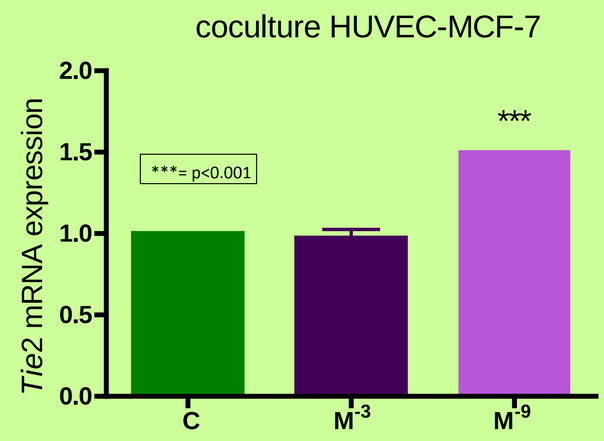
Melatonin at physiological doses (1 nM) increased both Ang1 and Ang2 gene expression in MCF-7. Pharmacological concentrations (1 mM) were also effective to increase Ang1 and Ang2 expression. In addition, physiological and pharmacological concentrations of melatonin increased *Ang 1* and *Ang2* in HUVEC. *Tie2* expression in HUVEC was also enhanced by melatonin at 1 nM and 1 mM but in coculture with MCF-7 only pharmacological doses of melatonin was able to increase mRNA expression.

Effects of melatonin on the expression of Ang-1, Ang-2 and Tie2 receptor in HUVEC









DISCUSSION

Ang-1 is a critical player in vessel maturation and it mediates migration, adhesion and survival of endothelial cells, but in breast cancer, it may not function as a promoter of angiogenesis, although it may act retarding tumor growth. Furthermore, Ang-1 and VEGF regulate angiogenesis at different levels, with VEGF inducing vessel sprouting and growth and Ang-1 mediating vessel remodeling and maturation. Ang1 acts in an agonistic manner inducing Tie2 phosphorylation and subsequent vessel stabilization. On the other hand, Ang-2 could antagonize the Ang1-mediated Tie2 activation. This angiopoietin induces tumor growth and invasiveness, but also reduces angiogenesis, promotes cell death and vascular regression. In addition to VEGF, Ang-2 promotes neo-vascularization. The receptor Tie 2 is essential for vascular maturation, but it is not required for the vasculogenic formation of endothelial cells. However, it is required for the proliferation and maintenance of these cells.

Since it is known that melatonin inhibits VEGF expression in MCF-7 cells and that an increase in angiopoietins expression in the absence of VEGF lead to vessel regression, our findings suggest the melatonin could inhibit angiogenesis through an up-regulation on angiopoietins expression and a down-regulation on VEGF expression.

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CONCLUSION

Melatonin behaves as an anti-angiogenesis molecule by increasing angiopoietins 1 and 2 mRNA expression and decreasing VEGF expression.

Thus, melatonin could be an effective therapeutic strategy to block tumor angiogenesis.

REFERENCES

1. Cos S. et al. Estrogen signaling pathway: A link between breast cancer and melatonin oncostatic actions. Cancer Detection and Prevention. 2006. 30:118-128.
2. Fagiani E. and Christofori G. Angiopoietins in angiogenesis. Cancer letters. 2013. 328: 18-26.
3. Hayes AJ et al. Expression and function of angiopoietin-1 in breast cancer. British Journal of Cancer. 2000. 83:1154-1160.
4. Thomas M. and Agustin H.G. The role of the Angiopoietins in vascular morphogenesis. Angiogenesis. 2009. 12:125-137.
5. Thurston G. and Daly C. The Complex Role of Angiopoietin-2 in the Angiopoietin-Tie signaling pathway. Cold Spring Harbor Perspectives in Medicine. 2012. 2:a006650.