Melatonin Inhibits the Migration of Human Lung Adenocarcinoma A549 Cell Lines Involving JNK/MAPK Pathway

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Abstract

Objective: Melatonin, an indolamine produced and secreted predominately by the pineal gland, exhibits a variety of physiological functions, possesses antioxidant and antitumor properties. But, the mechanisms for the anti-cancer effects are unknown. The present study explored the effects of melatonin on the migration of human lung adenocarcinoma A549 cells and its mechanism.

Methods: MTT assay was employed to measure the viability of A549 cells treated with different concentrations of melatonin. The effect of melatonin on the migration of A549 cells was analyzed by wound healing assay. Occludin location was observed by immunofluorescence. The expression of occludin, osteopontin (OPN), myosin light chain kinase (MLCK) and phosphorylation of myosin light chain (MLC), JNK were detected by western blots.

Results: After A549 cells were treated with melatonin, the viability and migration of the cells were inhibited significantly. The relative migration rate of A549 cells treated with melatonin was only about 20% at 24 h. The expression level of OPN, MLCK and phosphorylation of MLC of A549 cells were reduced, while the expression of occludin was conversely elevated, and occludin located on the cell surface was obviously increased. The phosphorylation status of JNK in A549 cells was also reduced when cells were treated by melatonin.

Conclusions: Melatonin significantly inhibits the migration of A549 cells, and this may be associated with the down-regulation of the expression of OPN, MLCK, phosphorylation of MLC, and up-regulation of the expression of occludin involving JNK/MAPK pathway.

Introduction

Lung cancer is the most common cancer and the leading cause of cancer deaths worldwide. The prognosis of patients can be improved through effective treatment, but the 5-year survival rate of the patients with advanced lung cancer is only about 16% [1]. Various novel therapeutic strategies currently under consideration as the clinical use of cytotoxic drugs is limited due to intrinsic or acquired resistant and toxicity [2]. The majority of patients with lung cancer presents with locally advanced inoperable or metastatic disease [3].

Cell migration is a biological process that contributes crucially to a variety of physiological, wound healing and the inflammatory reaction. Moreover, cell migration is also responsible for the malignance of cancer disease as it allows tumor cells to invade the surrounding tissues, thereby forming metastases [4].

Recent studies demonstrate that many proteins, such as myosin light-chain kinase (MLCK), osteopontin (OPN), play a critical role in non-muscle cell protrusion, contraction, and migration [5–6]. MLCK is a key Ca2+/Calmodulin (CaM)-dependent effector that is responsible for smooth muscle cell and non-muscle cell migration via phosphorylation of Ser19, Thr18 on myosin light chains (MLC), an event that facilitates myosin interaction with actin filaments [7]. MLCK expression reduction via antisense techniques is lead to rounding fibroblast cell, decreasing proliferation and attenuating chemoattractant-stimulated cell locomotion [8]. OPN undergoes extensive posttranslational modification, including phosphorylation, glycosylation and cleavage, resulting in molecular mass variants ranging from 25 to 75 kDa [9]. There is evidence suggesting that multiple signals may function in OPN-mediated tumor cell migration as inhibitors to phospholipase C/protein kinase C (PLC/PKC), mitogen activated protein kinase (MAPK), and PI3K could decrease OPN-induced migration [6].

Tumor cells, particularly in those cancers that manifest high metastatic potential, often exhibit loss of tight junctions (TJ). Occludin is a transmembrane protein of epithelial TJs, therefore its structure is relatively well characterized [10–12]. Down-regulation of specific TJ proteins has been shown to correlate...
with the staging, invasiveness and metastasis potential of epithelial cancers [13–15]. MAPK signaling pathway is able to modulate TJ paracellular transport by up-or down-regulating the expression of several TJ proteins and hence altering the molecular composition within TJ complexes [16].

These observations directly implicate that MLCK, OPN, occludin in the signaling pathways controls the non-muscle cell motility. However, the exact mechanistics during cancer cell migration remain poorly understood.

MAPK pathways play pivotal roles in cell proliferation, differentiation, and survival [17]. The closely related MAPK pathways are regulated through a series of phosphorylation steps in a three-component module: MAPKs are activated by MAPK kinases (MAPKK) on dual residues of threonine and tyrosine, and MAPKKs are in turn phosphorylated by MAPKK kinases (MAPKKK) on dual residues of serine/threonine. MAPKs have been divided into three main groups: the extracellular-regulated kinases 1/2 (ERK1/2 or MAPK p42/44), MAP 38, and the c-Jun N-terminal kinases (JNK) [18]. It has been reported that JNK is constitutively activated in several tumor cell lines and that the transforming actions of several oncogenes have been reported to be JNK dependent (based on dominant-negative approaches) [19]. Recently, more and more evidences indicate that JNK substrates, especially the non-nuclear proteins, also have wide-ranging functional roles in cell migration, axonal guidance, neurite formation and outgrowth, brain development, dendritic architecture and regeneration of nerve fibers after injury [20].

Melatonin is an indole bioactivator mainly secreted by the pineal gland. It has a wide range of reported biologic effects including antioxidative [21–26], anti-inflammatory and antitumor activities [27–34] and has generated considerable interest as a pharmacological compound with a wide range of therapeutic activities. Melatonin has also been shown to possess chemotherapeutic potential in human cancers and to be capable of modulating several signal transduction pathways associated with cell survival, proliferation, apoptosis and invasion [35–39]. Recent studies have reported that melatonin can inhibit tumor invasion through increasing adhesion by elevating E-cadherin and integrin expression [40] or modulating microfilament [41–42], and decreasing matrix metalloproteinases (MMPs) production [43]. Anti-invasion effect of melatonin has been shown in human mammary epithelial cancer MCF-7 cells [40–41,43] and MDCK cells [42].

However, it is unknown whether melatonin affects the migration and invasion of A549 cells via OPN, occludin, as well as MLCK and through which pathway. Therefore, the present study was undertaken to investigate the effect of melatonin on the migration and invasion of A549 cells. In addition, we also assess the expression of OPN, occludin, MLCK, and the function of JNK MAPK signal transduction pathway.

### Materials and Methods

#### Cell culture

Human lung adenocarcinoma cell line (A549) was purchased from ATCC and cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (TBD Science, Tianjin, China), 1 mmol/L glutamine, 100 U/mL penicillin and 10 mg/mL streptomycin (Ameresco, USA), at 37 °C, 5% CO₂.

#### Reagents

Melatonin was provided by School of Pharmacy, Anhui Medical University (Anhui, China). Dimethyl Sulfoxide (DMSO) was obtained from sigma Chemical (USA). Dulbeccos modified Eagles medium (DMEM) was purchased from Gibco BRL Life Technologies (USA). Phorbol-12-myristate-13-acetate (PMA) and SP600125 were obtained from Cayman Chemical (USA). Primary antibodies (anti-OPN, anti-occludin, anti-MLCK, anti-pMLC, anti-MLC, anti-pJNK, anti-jNK, anti-β-actin) were purchased from Santa Cruz Biotechnology (USA). All secondary antibodies were purchased from Millipore (USA). ECL reagent and BCA kit were purchased from quantitative Pierce Company.

#### Cell viability assay

Cell viability was measured using the MTT assay. A549 cells (4.5 × 10⁴ cells/well) were seeded into 96-well plates and cultured. The cells were treated with different concentrations of melatonin (0.1, 0.5, 0.75, 1.0, 2.5, 5.0 mmol/L), then incubated with MTT solution for 4 h. Finally, the cells were exposed to an MTT-formazan dissolving solution (DMSO) for 30 minutes. The optical density (OD) was measured using an absorbance microplate reader (Bio-Tek, ELX800) at a wavelength of 490 nm. The cell viability was expressed as a percentage of the OD value of the control cultures. For the A549 cells, the data were taken from one experiment with 4 replicates. IC₅₀ was determined using a sigmoidal equilibrium model regression using XLfit version 4.3.2 (ID Business Solutions Ltd.) and is defined as the concentration of melatonin required for a 50% reduction in growth/viability.

#### Wound healing assay

Migration of A549 cells was measured using the wound-healing assay in vitro. Cells were seeded into 12-well plates and grown to

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**Table 1.** The effect of different concentrations of MLT on the viability of A549 cells in 3 day.

| Group       | OD570 nm (Mean ± SD) | Inhibition rate (%) | IC50 (mmol/L) |
|-------------|----------------------|---------------------|---------------|
| DMSO        | 0.67±0.04            | –                   | 1.864         |
| MLT (mmol/L)|                      |                     |               |
| 0.10        | 0.55±0.05            | 17.50*              |               |
| 0.50        | 0.51±0.02            | 23.53*              |               |
| 0.75        | 0.47±0.02            | 29.00*              |               |
| 1.00        | 0.41±0.03            | 38.78*              |               |
| 2.50        | 0.18±0.02            | 72.99*              |               |
| 5.00        | 0.10±0.01            | 84.15*              |               |

Compared with DMSO control group, *P<0.05.

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Figure 1. Melatonin inhibits the migration of A549 cells. (A) The migration of A549 cells at 0.1, 0.75, 2.5, 5.0 mmol/L melatonin groups respectively when A549 cells were treated for 0 h, 12 h and 24 h. (B) Analysis of migration rate (%), compared with control group (DMSO): *P<0.05, #P<0.05.
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Figure 2. The effect of melatonin, SP600125 and PMA on migration of A549 cells. (A) The effect of melatonin, SP600125 and PMA on migration of A549 cells after 0 h, 12 h and 24 h. (B) Analysis of migration rate, compared with control group: *P<0.05, #P<0.05; compared with PMA group: ▲P<0.05, ▲P<0.05.
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100% confluence. Wounds were created by scraping monolayer cells with a sterile pipette tip. At 0, 12, 24 h after the creation of wounds, cells were observed with 10× objective in an Olympus (Olympus Corporation, Tokyo, Japan) photomicroscope. Images were acquired with a Nikon (Tokyo, Japan) color digital camera. Wound distances were measured at each time point and expressed as the average percent of wound closure by comparing the zero time.

Immunofluorescence assay
A549 cells (0.5×10⁴ cells/well) were seeded into 96-well plates with sterile aseptic cover glasses and cultured. Cells were treated with 0.1 mmol/L and 2.0 mmol/L concentration melatonin for 7 d. DMSO was added in the control group. The nutrient solution was changed every day. After treatment, the cells were washed and fixed with 4% paraformaldehyde for 20 minutes at room temperature, then washed and blocked with blocking buffer (5% nonfat dry milk in PBS) for 2 h at room temperature. The cover glass with A549 cells was incubated with goat anti-human occludin (1:50) primary antibody overnight at 4°C. Cells were washed and incubated with donkey anti-goat IgG-FITC (1:200), then washed and mounted with aqueous-based anti-fade mounting medium. Images of stained cells were captured using fluorescence microscope.

Western blotting analysis
After treatment, cells were washed with PBS for 3 times and lysed in lysis buffer (1% TritonX-100, 0.015 M NaCl, 10 mM Tris–HCl, 1 mM EDTA, 1 mM PMSF, 10 lg/mL of each leupeptin and peptastin A). The protein concentrations were measured with a BCA kit. The cell lysates were solubilized in SDS sample buffer, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to a polyvinylidene fluoride (PVDF) membranes. The membrane was blocked with blocking buffer (5% nonfat dry milk) overnight at 4°C. After that, the membrane was incubated with the indicated primary antibody with OPN(1:500), occludin(1:250), MLCK(1:500), pMLC(1:500), MLC(1:1000), pJNK(1:500), JNK(1:1000), and β-actin(1:1000) respectively and followed by the appropriate horseradish peroxidase(HRP)-conjugated secondary antibody, and visualized with enhanced chemiluminescence using hydrogen peroxide and luminol as substrate with Kodak X-AR film. The images were scanned using a ScanPrisa1240 OUT (Acer, China). Western blots data were quantified using Quantity One software.

Statistical analysis
Three or more separate experiments were performed for each experiment. Statistical analysis was performed by Student’s t-test or ANOVA. Data are presented as means ± standard deviation. Statistical significance was defined as p value less than 0.05.

Results

Effect of melatonin on viability in A549 cells
To determine the effect of melatonin on cell viability, A549 cells were treated with different concentrations of melatonin (0.1, 0.5, 0.75, 1.0, 2.5, 5.0 mmol/L) for 3 d. Viability was assessed using the MTT assay. The results showed that melatonin inhibited the proliferation of A549 cells in a concentration-dependent manner, compared with the control group. The inhibition rates were 17.50%, 23.53%, 29.00%, 38.78%, 72.99% and 84.15%, respectively (Table 1). The IC 50 of melatonin is 1.864 mmol/L.

Effect of melatonin on migration in A549 cells
To investigate the effect of melatonin on migration, A549 cells were treated with different concentrations of melatonin. Migration of A549 cells were inhibited by melatonin in a concentration-dependent manner (Fig. 1). We then used pharmacological inhibitors and activator to determine the role of JNK in the migration of A549 cells. The A549 cells were treated with SP600125 (JNK inhibitor) and PMA (MAPK activator) for 3 d. The results showed that SP600125 significantly inhibited the migration of A549 cells and PMA has no obvious effect on the migration of A549 cells compared with the control while PMA decreased the effects of Melatonin and SP600125 on migration of A549 cells (p<0.05) (Fig. 2).

Melatonin up-regulates the expression of occludin and enhances occludin to locate on the cell surface, and down-regulates the expression of OPN, MLCK in A549 cells, which is partly through the JNK/MAPK signaling pathway
Tight junction correlated protein occludin was detected by immunofluorescence in A549 cells. The results showed that there was no occludin accumulation on the A549 cells surface, but occludin started to locate on the cell surface when A549 cells was treated with melatonin at the concentration of 0.1 mmol/L. Occludin located on the cell surface was obviously increased when treated with melatonin at the concentration of 2.0 mmol/L (Fig. 3). The effect of melatonin on the expression of proteins related with...
migration was determined using western blots analysis in A549 cells. After the cells were treated with different concentrations of melatonin for 3 d, the results revealed that melatonin (2.0 mmol/L) enhanced the expression of occludin ($p<0.05$) (Fig. 4 A), reduced the expression of OPN (Fig. 4 B), MLCK and phosphorylation of MLC (Fig. 4 C), JNK (Fig. 4 D).

After that, we examined whether the expression of related proteins is associated with JNK/MAPK. In later western blots experiment, we then used pharmacological inhibitors and activator to determine the role of JNK in the expression of related proteins in A549 cells, the cells were treated with melatonin, SP600125, and PMA for 3 d. The phosphorylation status of JNK

Figure 4. Effect of melatonin on the expression of occludin, OPN, MLCK and phosphorylation of MLC, JNK. (A) occludin, (B) OPN, (C) MLCK and MLCK, (D) JNK. Results are presented as mean ± SD of three independent experiments. *$P<0.05$, in comparison to control group. doi:10.1371/journal.pone.0101132.g004
was decreased. The expression level of OPN, MLCK, and phosphorylation of MLC were down-regulated, while the expression of occludin was up-regulated when cells were exposed to melatonin and SP600125 compared with control group (p < 0.05). The expression level of OPN, MLCK, phosphorylation of MLC, JNK were lower in melatonin plus SP600125 group while the expression level of occludin was increased (p < 0.05) (Fig. 5).

Figure 5. The Effect of melatonin, SP600125 and PMA on the expression of occludin, OPN, MLCK and phosphorylation of MLC, JNK. (A) occludin, (B) OPN, (C) MLC and MLCK, (D) JNK. Results are presented as mean ± SD of three independent experiments. *P < 0.05, **P < 0.05, in comparison to control; *P < 0.05, **P < 0.05, in comparison to PMA group. doi:10.1371/journal.pone.0101132.g005

Discussion

Cell migration is a biological process that contributes crucially to a variety of physiological functions, such as wound healing and the inflammatory reaction. Moreover, cell migration is also responsible for the malignance of cancer as it allows tumor cells to invade the surrounding tissues, thereby forming metastases [4]. Melatonin can inhibit tumor cell proliferation, selectively blocking the signal transduction of tumor cells, and destroying its autonomous growth, which has become a research hot point
OPN has repeatedly been shown to be present at high levels in the circulation of patients with metastatic cancers [53–57] and increased metastatic potential [58–60], thus make it relevant in the context of studying its expression in the perspective of metastasis. Both activated myosin II and its activator MLCK are enriched in lamellipodial protrusive structures in several cell types during migration [61]. MLCK activation and expression have been found to be positively related with metastatic propensity [62–63]. Our results demonstrated that melatonin significantly suppressed the JNK/MAPK pathway in A549 cells. To determine the mechanism, we used PMA and SP600125 to respectively activate and inhibit MAPK/JNK signaling in A549 cells. Our results showed that SP600125 significantly inhibited the migration of A549 cells. Melatonin and SP600125 inhibited the relative migration rate of groups in PMA-stimulated groups. The expression of OPN and MLCK, the phosphorylated of MLC were down-regulated while the expression level of occludin was up-regulated when cells were exposed to SP600125. Melatonin and SP600125 inhibited the expression of MLCK, OPN, and phosphorylated of JNK, MLC, and enhance the expression of occludin in PMA-stimulated groups. In accordance with our data, therefore, the anti-migration effect of melatonin is associated with its inhibition of JNK/MAPK pathway and regulation of the expression of occludin, OPN, and MLCK.

Our present results show that melatonin plays an important role in inhibiting the proliferation and migration of A549 cells. Occludin, OPN, and MLCK contribute to the migration of A549 cells involving JNK/MAPK pathway. Our findings support the potential application of melatonin in the treatment of lung cancer. Due to the migration of tumor cells and the formation of tumor metastasis is an extremely complex process which involves multiple steps and many factors, the comprehensive mechanism of melatonin inhibits tumor cell migration still needs further research.

**Author Contributions**

Conceived and designed the experiments: YW SG. Performed the experiments: Qiaoyun Zhou Qing Zhou YW SG. Analyzed the data: Qiaoyun Zhou Qing Zhou YW SG. Contributed reagents/materials/analysis tools: Qiaoyun Zhou Qing Zhou YW SG. Wrote the paper: Qiaoyun Zhou SG YW.
Melatonin Inhibits the Migration of A549 Cells

19. Ip YT, Davis RJ (1998) Signal transduction by the c-Jun N-terminal kinase (JNK)-from inflammation to development. Current Opinion in Cell Biology 10: 205–219.

20. Bogunovic M, Kobe B (2006) Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. Microbiology and Molecular Biology Reviews 70: 1061–1095.

21. Domínguez-Rodríguez A, Breu-González P (2011) Melatonin: still a forgotten antioxidant. International Journal of Cardiology 149: 382.

22. Korkmaz A, Reiter RJ, Topal T, Manchester LC, Oter S, et al. (2009) Melatonin: an established antioxidant worthy of use in clinical trials. Molecular Medicine 15: 43–50.

23. Galano A, Tan DX, Reiter RJ (2011) Melatonin as a naturally against oxidative stress: a physicochemical examination. Journal of Pineal Research 51: 1–16.

24. Bonnemort-Rousselot D, Collin F, Jore D, Gardès-Albert M (2011) Reaction mechanism of melatonin oxidation by reactive oxygen species in vitro. Journal of Pineal Research 50: 320–335.

25. Reiter RJ, Tan DX, Poeggeler B, Menendez-Pelaez, Chen L, et al. (1994) Melatonin as a free radical scavenger: implications for aging and age-related diseases. Annals of The New York Academy of Sciences 719: 1–12.

26. Tan DX, Chen LD, Poeggeler B, Manchester LC, Reiter RJ, et al. (1993) Melatonin: a potent endogenous hydroxyl radical scavenger. Endocrine Journal 1: 57–60.

27. Hill SM, Blask DE, Xiang S, Yuan L, Mao L, et al. (2009) Melatonin and its relation with the psychospiritual status of cancer patients. Journal of Research in Medical Sciences 15: 225–228.

28. Messina G, Lissoni P, Marchiori P, Bartolacelli E, Brivio F, et al. (2010) Enhancement of the efficacy of cancer chemotherapy by the pineal hormone melatonin and its relation with the psychosomatic status of cancer patients. Journal of Research in Medical Sciences 15: 225–228.

29. Padillo EF, Ruiz-Rabelo JF, Cruz A, Perea MD, Tassell I, et al. (2010) Melatonin and celecoxib improve the outcomes in hamsters with experimental pancreatic cancer. Journal of Pineal Research 49: 264–270.

30. Grant SG, Melan MA, Latimer JJ, Witt-Enderby PA (2009) Melatonin and vitamin D3 synergistically down-regulate Akt and MDM2 expression of v-erb-B2, avian erythroblastosis virus-transformed NIH 3T3 cells. Anticancer Research 29: 195–205.

31. Jung-Hynes B, Reiter RJ, Ahnand N (2010) Sinrtains, melatonin and circular rhythm: building a bridge between aging and cancer. Journal of Pineal Research 48: 9–19.

32. Gonzalez A, Del Castillo-Vaquero A, Miro-Moran A, Tapia JA, Salido GM, et al. (2011) Melatonin reduces pancreatic tumor cell viability by altering mitochondrial physiology. Journal of Pineal Research 50: 250–260.

33. Um HJ, Park JW, Kwon TK (2011) Melatonin sensitizes Caki renal cancer cells to kalrenon-induced apoptosis through CHOP mediated up-regulation of ER stress. Journal of Pineal Research 50: 159–166.

34. Mao L, Yuan L, Slayek LM, Jones FE, Burow ME, et al. (2010) Inhibition of breast cancer cell invasion by melatonin is mediated through regulation of the p38 mitogen-activated protein kinase signaling pathway. Breast Cancer Research 12: R107.

35. Proietti S, Casicina A, D’Anselmi F, Dinicola S, Pasqualato A, et al. (2011) Melatonin and vitamin D3 synergistically down-regulate Akt and MDM2 leading to TGFbeta-1-dependent growth inhibition of breast cancer cells. Journal of Pineal Research 50: 150–156.

36. Martinez-Campa CM, Alonso-González C, Mediavilla D, Cos S, González A, et al. (2008) Melatonin down-regulates kERT expression induced by either natural estrogens (17beta-estradiol) or metalloestrogens (cadmium) in MCF-7 human breast cancer cells. Canceier Letters 268: 272–277.

37. Dai M, Cui P, Yu M, Han J, Li H, et al. (2008) Melatonin modulates the expression of VEGF and HIF-1 alpha induced by CoCl2 in cultured cancer cells. Journal of Pineal Research 44: 121–129.

38. Cos S, Fernández R, Guzmán A, Sánchez-Barceló EJ (1998) Influence of melatonin on invasive and metastatic properties of MCF-7 human breast cancer cells. Cancer Research 58: 4383–4390.

39. Ortiz-Lopez L, Morales-Mullas S, Ramirez-Rodriguez G, Benitez-King G (2009) ROCK-regulated cytoskeletal dynamics participate in the inhibitory effect of melatonin on cancer cell migration. Journal of Pineal Research 46: 15–21.

40. Ramirez-Rodriguez G, Oriz-Lopez L, Benitez-King G (2007) Melatonin increases stress fibers and focal adhesions in MDCK cells: participation of Rho-associated kinase and protein kinase C. Journal of Pineal Research 42: 180–190.

41. Bellon A, Oriz-Lopez L, Ramirez-Rodriguez G, Anton-Tay F, Benitez-King G (2007) Melatonin induces neurogenesis at early stages in NE1-113 cells through actin rearrangements via activation of protein kinase C and Rho-associated kinase. Journal of Pineal Research 42: 214–221.

42. Tam CW, Mo CW, Yao KM, Stephen YW, Shin SY (2007) Signaling mechanisms of melatonin in anti-proliferation of hormone-refractory 22Rv1 human prostate cancer cells: implications for prostate cancer chemoprevention. Journal of Pineal Research 42: 191–202.

43. AL-Sherief BF, Shalata HR, Sampali J, Jothy S (1995) Prognostic significance of proliferating cell nuclear antigen expression colorectal cancer. Cancer 71: 1954–1959.

44. Britz-King G, Soto-Vega E, Ramirez-Rodriguez G (2009) Melatonin modulates microfilament phenotypes in epithelial cells: implications for adhesion and inhibition of cancer cell migration. Histology and Histopathology 24: 789–796.

45. Fornas O, Mato ME, Webb SM (2000) Anti-proliferative effect and cell cycle modulation by melatonin on GH (3) cells. Hormone Research in Paediatrics 53: 251–255.

46. Messina GD, Cos S, Sánchez-Barceló EJ (1999) Melatonin inhibits p53 and p21WAF1 expression in MCF-7 human breast cancer cells in vitro. Life Sciences 63: 415–420.

47. Tobioka H, Isomura H, Kokai T, Tokumaga Y, Yamaguchi J, et al. (2004) Osteopontin expression decreases concomitantly with the progression of human endometrial carcinoma. Human Pathology 35: 159–164.

48. Kimura Y, Shiozaki H, Hirao M, Maeno Y, Doki Y, et al. (1997) Expression of occludin, tight junction associated protein, in human digestive tract. American Journal of Pathology 151: 45–54.

49. Davies DC (2002) Blood-brain barrier breakdown in septic encephalopathy and brain tumor. Journal of Anatomy 200: 639–646.

50. Papadopoulos MC, Saadoun S, Woodrow CJ, Davies DC, Costa-Martinus P, et al. (2003) Oocludin expression in microvesicles of neoplastic and non-neoplastic human brain. Neuropathology and Applied Neurobiology 27: 384–395.

51. Bramwell VH, Doig GS, Tuck AB, Wilson SM, Tonkin KS, et al. (2006) Serial plasma osteopontin levels have prognostic value in metastatic breast cancer. Clinical Cancer Research 12: 3357–3343.

52. Hutz SJ, Winquist EW, Stitt L, Chambers AF (2002) Plasma Osteopontin. Cancer 95: 506–512.

53. Fedarko NS, Jain A, Karadag A, Van Eman MR, Fisher LW (2008) Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. Clinical Cancer Research 7: 4060–4066.

54. Bramwell VH, Tuck AB, Wilson SM, Stitt L, Chambers AF, et al. (2005) Expression of osteopontin and HGF in metastatic prostate tumors. The American Journal of Pathology 166: 1671–1679.

55. Bramwell VH, Tuck AB, Wilson SM, Stitt L, Cherian AK, et al. (2005) Expression of osteopontin and HGF in metastatic prostate tumors. Molecular Cancer Therapy 4: 1336–1341.

56. Chambers AF, Behrendt EJ, Wilson SM, Denhardt DT (1992) Inhibition of expression of osteopontin (osteopontin; secreted phosphoprotein) in metastatic, ras-transformed NIH 3T3 cells. Anticancer Research 12: 43–47.

57. Oates AJ, Baraclough R, Rudland PS (1996) The identification of osteopontin as a metastasis-related gene product in a rodent mammary tumour model. Oncogene 13: 97–106.

58. Ramankutav A, Lein M, Kristiansen G, Loeving SA, Jung K (2007) Plasma osteopontin in comparison with bone markers as indicator of bone metastasis and survival outcome in patients with prostate cancer. The Prostate 67: 330–340.

59. Kolega L (2003) Asymmetric distribution of myosin IIB in migration endothelial cell. Human Pathology 34: 205–219.

60. Chamber AF, Behrendt EI, Wilson SM, Denhardt DT Vascular endothelial growth factor (VEGF) and survival outcome in patients with prostate cancer. The American Journal of Pathology 151: 45–54.

61. Chambers AF, Behrendt EI, Wilson SM, Denhardt DT Vascular endothelial growth factor (VEGF) and survival outcome in patients with prostate cancer. The American Journal of Pathology 151: 45–54.