Differential expression of Ran-binding protein 2 (RanBP2) and Targeting Protein for Xklp2 (TPX2) in head and neck squamous cell carcinoma cells

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ABSTRACT

RanGTPase is overexpressed in various human cancer cell lines. RanGTPase-related genes are essential for viability of head and neck cancer cell lines. However, it is currently unclear whether RanGTPase play a role in metastasis of head and neck cancer. This study aimed to examine mRNA and protein expressions of RanGTPase in primary and metastatic head and neck cancer cell lines using real-time PCR and Western blot analyses. Three pairs of head and neck cancer cell lines were used. HN18, HN30 and HN4 were derived from primary lesions, whereas HN17, HN31 and HN12 were derived from metastasis lesions from to same patients, respectively. The results were compared to those obtained from HaCat, an immortalized non-tumorigenic human keratinocyte cell line. Cells were cultured and total RNA was extracted and converted to complementary DNA. Expression at the mRNA level of *Ran*, *KPNB1*, *RanBP2*, and *TPX2* was monitored using real-time PCR. *GAPDH* was used as an internal control. Western blotting was also performed to examine the protein expression of Ran, Importin-beta, RanBP2 and TPX2. Real-time PCR data showed that mRNA expression of *Ran*, *KPNB1*, *RanBP2* and *TPX2* in primary and metastatic head and neck cancer cell lines was significantly different from that of HaCat at p value of 0.0079, 0.0005, 0.0074 and 0.0039, respectively. Furthermore, expression of mRNA transcripts of *Ran* and *TPX2* in the metastatic head and neck cancer cell lines was higher than that of primary cancer cells. However, protein expression of RanBP2 and TPX2 was found to be higher in the metastatic cancer cells compared to their primary counterparts. These findings suggest that expressions of mRNA transcripts and proteins of RanGTPase network were significantly different from those of normal cells. Particularly, RanBP2 and TPX2 expressions was elevated in the metastatic head and neck cancer cell lines. Further studies are required to understand its functional role in metastatic head and neck cancer.

Keywords: head and neck cancer; RanGTPase; RanBP2; TPX2

INTRODUCTION

Head and neck cancer accounts for approximately ten per cent of all human cancers and about 40% of them occur in the oral cavity. More than 90 percent of oral cancer is squamous cell carcinoma. Head and neck squamous cell carcinoma (HNSCC) is a devastating disease with high morbidity and mortality rates and with a moderate to high prevalence in certain parts of the world, including Thailand (Iamaroon *et al*., 2004). Only 40-50% of patients diagnosed with HNSCC will be able to survive for five years. Moreover, most cases are detected in the advanced cancer stage with lymph node metastases. About 50% of patients with locally advanced HNSCC fail to respond to standard therapies and develop recurrences and distant metastases (Argiris *et al*., 2008, Leemans *et al*., 2011). Currently, limited curable therapeutics is available for HNSCC patients with recurrent/metastatic disease. In addition, understanding how cancer becomes invasive and metastatic may lead to a more effective treatment of the disease as certain differential gene expression in primary HNSCC can predict the presence of lymph node metastases (Roepman *et al*., 2005).

The small GTPase Ran (RanGTPase), a member of Ras superfamily of small GTPases, has recently emerged to play a role in cancer cell proliferation and metastasis. A genome-wide siRNA screen suggested that the RanGTPase plays a functional role in cellular survival in head and neck cancer cell lines (Martens-de Kemp *et al*., 2013). Multiple genes are known to participate in the RanGTPase signaling. The candidate genes whose function were associated with several cancer types include Ran, KPNB1, TPX2 and Ran-binding protein 2 (RanBP2). A recent study also indicated the anti-tumor effects of KPNB1 in the genome-wide CRISPR/Cas9 screen (Kodama *et al*., 2017). Indeed,

KPNB1 levels were found to be over-expressed in many cancer types, such as cervical cancer (van der Watt *et al*., 2009), hepatocellular carcinoma (Yang *et al*., 2015) and glioma (Lu *et al*., 2016). RanBP2 is a nucleoporin with SUMO E3 ligase activity. Functionally, RanBP2 plays a role as a negative regulator of cancer cell proliferation as a tumor suppressor gene (Dawlaty *et al*., 2008). In addition, overexpression of Ran was also detected in several cancer types, such as breast cancer (Ly *et al*., 2010) and colon cancer (Fan *et al*., 2013). Ran is regulated through the interaction with Survivin which is a regulator of mitosis overexpressed in tumor cells (Xia *et al*., 2008a). Disruption of the Ran-Survivin interaction resulted in defective spindle assembly factor TPX2 localization at microtubules (Xia *et al*., 2008a). Importantly, repression of Ran expression caused cancer cell death due to defective TPX2 functional role for mitotic spindle formation, although the normal cells remained unharmed (Xia *et al*., 2008a, Xia *et al*., 2008b). TPX2 has also been reported to overexpress in many types of cancer cells and tissues (Chang *et al*., 2012, Liu *et al*., 2015, Warner *et al*., 2009, Wei *et al*., 2013). TPX2 was shown to be associated with poor survival rate of patients with colon and ovarian cancers (Caceres-Gorriti *et al*., 2014, Wei *et al*., 2013). Moreover, functional studies showed that TPX2 was required for metastasis in colon and liver cancer (Liu *et al*., 2015, Wei *et al*., 2013). Together, the RanGTPase signaling cooperatively participates in survival and metastatic potential of several types of cancer. However, little is known how RanGTPase-associated proteins are regulated in metastatic cancer of HNSCC.

This study used three pairs of head and neck cancer cell lines. Each pair represents primary and the lymph node metastatic cancer cell lines isolated from the same patient (Cardinali *et al*., 1995). The head and neck cancer cell lines are of epithelial cell type origin. Moreover, the transcriptomic and proteomic data have been previously characterized in HNSCC cell lines (Cardinali *et al*., 1995, Koontongkaew *et al*., 2009, Perez-Ordonez *et al*., 2006, Squire *et al*., 2002). A recent study has also showed that numerical alterations of chromosomes were present in HNSCC cell lines. Interestingly, structural alterations were frequently found in metastatic cell lines. The objective of the present study was to investigate expression of candidate genes of RanGTPase, including Ran, KPNB1, TPX2 and RanBP2 in terms of both expression of mRNA transcript and protein in three pairs of HNSCC cell lines HN18/ HN17, HN30/HN31 and HN4/HN12. The mRNA and protein expressions were analyzed by quantitative real-time PCR and Western blotting, respectively.

MATERIALS AND METHODS

Cell culture

The three pairs of HN cell lines were established from 3 individuals with different clinical stages according to TNM staging system. The three pairs of cell lines HN18/HN17, HN30/HN31 and HN4/HN12 cell lines were classified as T2N2M0, T3N0M0, T4N1M0, respectively (Cardinali *et al*., 1995). Three pairs of HNSCC cell lines HN18/ HN17, HN30/HN31 and HN4/HN12 were kindly provided by Prof. J. Silvio Gutkind and used under supervision of Prof. Sittichai Koontongkaew. HN18 HN30, HN4 were derived from primary lesions whereas HN17 HN31, HN12 were derived from metastatic lymph node lesions which belong to same patients, respectively. The non-tumorigenic skin keratinocyte cell line HaCaT was originally provided by Prof. Stitya Sirisingha and used as a control cell line to examine the gene expression. All cell lines were cultured in Dulbecco's Modified Eagle Medium (Life Technologies-Gibco, USA) supplemented with 10% fetal bovine serum (Life Technologies-Gibco), 100 mg/ml penicillin—streptomycin (Life Technologies-Gibco), 100 mg/ml Amphotericin B. Cell lines were maintained in the CO_2 incubator under 95% humidified atmosphere at 37°C. This study was approved by the Institute Biosafety Committee of Thammasat University (TU-IBC, Approval No. 005/2558).

RNA isolation and cDNA synthesis

Total RNA was isolated from all cell lines using TRIzol® reagent (Life Technologies, USA) according to the manufacturer's protocol briefly as follows. Cells were washed in PBS and lysed with TRIzol® reagent. Cell pellet was collected using centrifugation at 12,000 g, 4°C for 10 minutes. Chloroform and isopropanol were used for isolation of RNA pellet. The pellet was then washed in 75% ethanol and dissolved in DEPC-treated water. The quantity and quality of RNA was measured with Nanodrop 2000 Spectrophotometer (Thermo Scientific, USA). Five hundred ng of total RNA was used for each cDNA synthesis reaction. DNA fragments were synthesized using iScript™ cDNA Synthesis Kit (Bio-Rad, USA) according to the manufacturer's protocol.

Real-time PCR

The mRNA expression of *Ran*, *KPNB1*, *RanBP2* and *TPX2* was measured by Bio-Rad iCycler

Thermal Cycler with iQ5 Multicolor Real Time PCR Detection System (Bio-Rad) as signal reporter. Fifty nanograms of cDNA was amplified in 10 µl of master mix containing 2X iTaq™ Universal SYBR® Green supermix (Bio-Rad), and 10.0 µM gene-specific primers (Table 1), designed from either the NCBI reference sequence or as indicated of *Ran (*NM_006325.4), *KPNB1* (NM_002265.5), RanBP2 (NM_006267.4) and TPX2 (Warner *et al*., 2009) genes. PCR conditions were as follows: an initial denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 15 s, 56-62°C for 30 s and 95°C for 1 min. Dissociation melting curves, created by running a heat dissociation protocol after the PCR (81 cycles of 55.0°C–95.0°C for 10 s, increase set point temperature by 0.5°C), confirmed the specificity of the amplicons and the absence of nonspecific products. The *GAPDH* gene was used as the internal reference to normalize the expression levels between samples. Quantitative real-time PCR reactions of all cell lines were performed in biological triplicates. Threshold cycle (Ct) values were used to calculate ΔCt values from the products of *GAPDH* and *Ran*, *KPNB1*, *RanBP2*, and *TPX2* genes for all cell lines. The relative expression levels of different cell lines were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

Western Blot

Head and neck cancer cell lines were washed in cold phosphate-buffered saline, lysed with RIPA buffer [1% Nonidet P-40, 0.5% deoxycholic acid sodium salt, 0.1% sodium dodecyl sulfate (SDS) and phosphate-buffered saline (pH 7.4)] supplemented with 10 mM sodium orthovanadate, 1 mM phenylmethanesulfonyl fluoride and 1 × proteinase inhibitor (Complete mini EDTA-free; Roche Applied Science, Indianapolis, IN, USA). Cell debris was removed by centrifugation at 10,000 ***g*** for 10 min. Protein content was determined using the Bicinchoninic Acid Protein Assay Kit (Pierce Biotechnology, Rockford, IL, USA). Equal masses of whole-cell protein were separated by electrophoresis on a 10% SDS–polyacrylamide gel (6% gel for RanBP2 blot) and transferred to a nitrocellulose membrane. The membrane was blocked with 5% bovine serum albumin or 5% semi-skimmed milk in Tris-buffered saline containing 0.05% Tween 20 (1 h, room temperature) and incubated overnight at 4°C with antibodies against anti-mouse Ran (BD, 1:1000) anti-mouse RanBP2 (D-4) (Santa Cruz Biotechnology, USA, 1:500) anti-rabbit TPX2 (Novus Biologicals, USA, 1:5000) anti-mouse Importin-β (BD, USA, 1:1000) and anti-mouse actin (Sigma, USA, 1:5000) followed by incubation in the same buffer with appropriate horseradish peroxidase-conjugated secondary antibodies (1 : 5,000 dilution; GE Healthcare, USA) for 1 h at room temperature. Immunoreactive proteins that were resolved to size as a band by gel electrophoresis were detected using enhanced chemiluminescence (Pierce Biotechnology) and exposed in the high-performance chemiluminescence system (GeneGnome; Syngene, Frederick, MD, USA). The image series were captured using exposure times varied between 1 and 20 min. Western blotting of β-actin was used as a loading control.

Statistical analyses

Statistical differences of *Ran*, *KPNB1*, *RanBP2*, and *TPX2* genes between each pair of cell lines were detected by one-way analysis of variance (ANOVA) with Turkey's multiple comparison test, using GraphPad Prism V5 (GraphPad Software, La Jolla, CA, USA), and the level of statistical significance was tested and represented as * for $P \leq 0.05$, Estimated values were expressed as mean ± standard error.

Table 1 Primer sequences for real-time quantitative reverse transcription PCR.

Gene	Primer	Sequence (5' → 3')
Ran	*Ran* Forward	GTT GTG TGG CAA CAA AGT GG
	Ran Reverse	AAA GCT GGG TCC ATG ACA AC
KPNB1	*KPNB1* Forward	TGT ACA GCA TTT GGG AAG GAT GT
	KPNB1 Reverse	TCG ATC TCC GCC CTT CAG T
RanBP2	*RanBP2* Forward	CTG ATG AAG ACA ATG GAA ATG GGG
	RanBP2 Reverse	CCA TCA CTT CAG TCC CAC CT
TPX2	*TPX2* Forward	CGA AAG CAT CCT TCA TCT CC
	TPX2 Reverse	TCC TTG GGA CAG GTT GAA AG
GAPDH	*GAPDH* Forward	GTC AAC GGA TTT GGT CGT ATT G
	GAPDH Reverse	CAT GGG TGG AAT CAT ATT GGA A

RESULTS

Differential mRNA expression of *Ran, KPNB1, RanBP2* and *TPX2* in paired head and neck squamous cells cancer cells

We first examined mRNA expression of RanGTP-associated genes, *Ran*, *KPNB1*, *RanBP2* and *TPX2*, in three pairs of HNSCC cells HN18/HN17, HN30/HN31 and HN4/HN12 in comparison with that of non-tumorigenic keratinocyte cell line HaCaT. Overall, differential expression of all four genes studied was found in head and neck cancer cells compared to that of HaCaT cells (Fig.1). The mRNA expression of *KPNB1*, *Ran* and *RanBP2* was highly elevated in the primary cancer cell HN18 compared to that of its metastatic counterpart cells HN17 (Fig.1A-C). The expression level of *KPNB1* greatly increased in the HN18 cell line whereas the expression almost disappeared in the metastatic counterpart HN17 cell line. However, differences of *KPNB1* expression between the primary and metastatic cells were not observed in HN30/HN31 and HN4/HN12 lines (Fig.1A). Similarly, the expression level of *Ran* substantially increased in HN18 compared to that of its metastatic cells HN17. Downregulation of *Ran* was found in HN30 and HN31 cell lines. Interestingly, the expression level of *Ran* in the metastatic cells HN31 significantly increased compared to that of the primary cancer cells HN30 (Fig.1B). The same pattern of the expression level in all cell lines was observed for the *RanBP2* gene. The expression level of *RanBP2* greatly elevated in HN18 cells compared to that of the metastatic cells HN17. However, an increase in the expression of *RanBP2* was found in the metastatic counterpart cells HN31 compared to that of the primary cancer cells HN30 (Fig.1C). There were no significant changes in the mRNA expression levels of *Ran* and *RanBP2* between HN4 and HN12 cell lines (Fig.1B, Fig.1C). The mRNA expression data of TPX2 showed the most consistent pattern. Two pairs of head and neck cancer cell lines HN18/HN17 and HN30/HN31 exhibited significantly higher expression in the metastatic cell line HN17 and HN31 compared with that of the primary counterpart HN18 and HN30, respectively. However, the mRNA level of *TPX2* in the metastatic cell line HN12 was downregulated compared to that of the primary cancer cell line HN4 (Fig.1D).

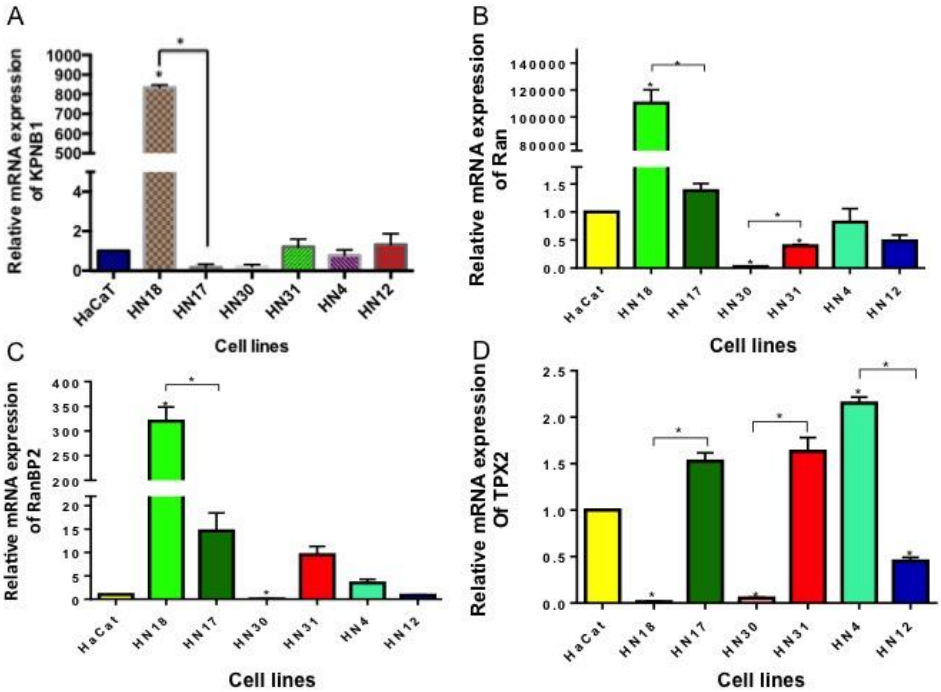

Figure 1. Relative mRNA expression of *KPNB1* (A), *Ran* (B), *RanBP2* (C) and *TPX2* (D). The mRNA expression levels were normalized with the expression of the housekeeping gene *GAPDH* and calculated as fold change compared to that of the control cell line HaCaT. The data were obtained from three independent experiments. One-way ANOVA was used to analyze the statistical differences. Asterisks indicate statistical significance at $P<0.05$.

RanBP2 and TPX2 proteins showed higher degree of expression in the metastatic head and neck cancer cells

The protein expression of Importin-β (*KPNB1* gene), Ran, RanBP2 and TPX2 were examined in three pairs of head and neck cancer cell lines HN12/HN4, HN31/HN30 and HN17/HN18, which represent cancer cells of the metastatic lymph nodes and the primary oral lesions, respectively. Any changes of the protein expression levels in the metastatic cell lines, therefore, were not due to the genetic background of the cell lines. Western blots showed unchanged levels of protein expression of Ran and Importin-β across three pairs of head and neck cancer cell lines in this study (Fig.2). Interestingly, TPX2 was found to be highly expressed in the metastatic cell line HN17 compared to that of the primary counterpart cells HN18. In addition, the expression level of TPX2 was also pronounced in the metastatic cell line HN31 compared to its primary cell line HN30, albeit to a much lower level than in the HN17/HN18 pair (Fig.2). It is to be noted that higher protein expression of TPX2 in the metastatic metastatic cell line HN31 compared to its primary cell line HN30, albeit to a much lower level than in the HN17/HN18 pair (Fig.2). The higher protein expression of TPX2 in the metastatic cells was consistent with the previous data of the mRNA expression (Fig.1D). Changes in the protein expression of RanBP2 were also found. Similar to TPX2, higher expression of RanBP2 was detected in the metastatic cell line HN17 compared to its primary cell line HN18 (Fig.2). The changes in the expression were not due to the different amount of total proteins detected by the loading control Actin (Fig.2). Taken together, differential expression of TPX2 and RanBP2 in the metastatic cell lines was detected at both mRNA and protein levels.

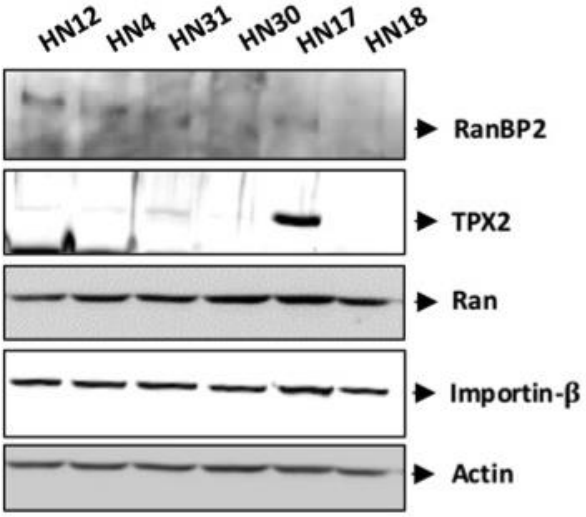

Figure 2. Western blots detected protein expression of RanBP2, TPX2, Ran, Importin-β in three pairs of head and neck cancer cell lines HN12/HN4, HN31/HN30 and HN17/HN18. Actin was used as a loading control.

DISCUSSION

RanGTPase is a member of Ras superfamily GTPase and plays important roles throughout cell division cycle, including nuclear transport of macromolecules during interphase and mitotic spindle formation and nuclear envelope reformation during mitotic cell division. Defects in RanGTPase signaling would, therefore, affects these crucial cellular activities (Clarke & Zhang, 2001, Kalab & Heald, 2008, Rensen *et al*., 2008).

The present study investigated the expression of candidate genes of RanGTPase, including *Ran*, *KPNB1*, *RanBP2* and *TPX2* in HNSCC cell lines. All genes tested showed differential expression from non-tumorigenic keratinocyte cell line HaCaT. RanGTPase-related genes are necessary for nucleocytoplasmic transport of RNA, tumor suppressor genes, oncogenes and transcription factors etc. (Rensen *et al*., 2008). Therefore, differential levels of RanGTPase genes in HNSCC cells found in this study may lead to dysregulation of tumor suppressor genes and oncogenes such as p53 and PTEN etc. in cancer cells (Rensen *et al*., 2008). Such molecules are required for proper regulation of cell division and detection of cells with serious mutations (Stadler *et al*., 2008). Failure of these control mechanisms would result in uncontrollable cell division and production of cells with genetic mutations. Accumulation of defective cells over time, therefore, induces tumorigenesis (Rensen *et al*., 2008).

The present study shows differential expression of RanGTPase in head and neck cancer cell lines compared to that of non-tumorigenic keratinocyte cells. Specifically, mRNA expression of *KPNB1*, *Ran* and *RanBP2* significantly increased only in the HN18 cell line which is the primary cancer cells derived from the base of tongue (Fig.1). This may be caused by tumor heterogeneity of head and neck cancer. Each pair of cells lines represents different oral lesions where the head and neck cancer cells arose from different individual (Cardinali *et al*., 1995). Indeed, distinct expression patterns at different anatomical sites in the oral cavity were reported previously in oral cancer (Frohwitter *et al*., 2017, Severino *et al*., 2008). However, Western blot result showed that only RanBP2 and TPX2 levels increased in the metastatic cells compared to that of the primary cells isolated from the same patient (HN12/HN4, HN31/HN30, HN17/HN18 (Fig.2). This finding was also consistent with a number of studies which showed differential expression between metastatic and non-metastatic HNSCC (Mendez *et al*., 2002, Mendez *et al*., 2007, Roepman *et al*., 2005, Ziober *et al*., 2006). The discrepancies between gene expression and protein expression could be due to post-translational modifications of protein, protein stability and lower rate of mRNA transcription in comparison with protein translation (Vogel & Marcotte, 2012). The protein expression result in this study suggests that both RanBP2 and TPX2 may play a role in the metastatic process of head and neck cancer cells. It is well understood that Ran, KPNB1, RanBP2 and TPX2 play an important role in the nuclear transport of macromolecules, mitotic spindle formation and nuclear envelop formation (Caceres-Gorriti *et al*., 2014, Clarke & Zhang, 2001, Kalab & Heald, 2008). Therefore, an increase in the expression of RanBP2 and TPX2 may indicate a necessity of both proteins in these crucial cellular activities in the metastatic head and neck cancer cells. The nucleocytoplasmic transport of specific macromolecules such as oncoproteins in the cancer cells may over-activate to enhance their proliferation capacity and invasiveness (Gama-Carvalho & Carmo-Fonseca, 2001, Hill *et al*., 2014). The discrepancy between the expression levels *in vitro* and *in vivo* was previously mentioned by a study of C-X-C chemokine receptor type 4 (CXCR4) in head and neck cancer cells. This study showed that there was no change in the expression levels of *CXCR4* in head and neck cancer cells between primary and metastatic cancer cells. However, the upregulation of *CXCR4* mRNA levels was found in the metastatic cancer tissue samples compared to that of the primary cancer tissues (Koontongkaew *et al*., 2012). It is possible that the expression of KPNB1, Ran and RanBP2 in head and neck cancer cells may be different in the in vitro study from the *in vivo* study due to an influence of tumor microenvironment (Koontongkaew *et al*., 2012).

Upregulation of Ran was detected in the metastatic cell line HN31 compared to that of the primary HNSCC cell line HN30 (Fig.1). This observation was consistent with the study that the expression of Ran was elevated in the metastatic renal cell carcinoma compared to that of its primary lesion (Abe *et al*., 2008). Functionally, Ran was also necessary for invasiveness in pancreatic cancer (Deng *et al*., 2014). Importantly, this study also uncovered that Ran was associated with invasive capacity via Androgen receptor and CXCR4 (Deng *et al*., 2014). The latter was also one of the important targets for metastatic phenotype in head and neck cancer. A study was found that CXCR4 induced the expression of *MMP*-9 and *MMP-13* in the tongue squamous cell carcinoma cells (Yu *et al*., 2011). Indeed, CXCR4 level was elevated in the metastatic head and neck cancer cells (Albert *et al*., 2013). However, the protein expression of Ran did not exhibit higher level of expression in the metastatic cells compared to its primary counterpart. Further studies are required to fully understand the metastatic role of Ran in head and neck cancer.

Increased expression of genes indicates a functional significance in cells. In this study, upregulation of TPX2 and RanBP2 in the metastatic head and neck cancer cells may indicate that they may serve some functional role in metastasis. Indeed, roles of TPX2 and RanBP2 on cancer metastasis has been studied. TPX2 was required for proliferation, migration and invasion in colon cancer and hepatocellular carcinoma (Liu *et al*., 2014, Wei *et al*., 2013). Interestingly, MMP-2 and MMP-9 were found to be linked with invasive function of TPX2 (Liu *et al*., 2014). It should be noted that both MMP-2 and MMP-9 are required for invasiveness in head and neck cancer (Rosenthal & Matrisian, 2006). RanBP2 is also a SUMO E3 ligase. RanBP2 was identified among several genes to be required for multiple myeloma tumorigenesis. The expression of RanBP2 was elevated in patient samples (Felix *et al*., 2009). More recently, understanding of the molecular mechanism of one of the major anti-cancer targets the insulin-like growth factor receptor (IGF-1R) revealed that sumoylation of IGF-1R required the interaction with RanBP2 in order to translocate into the nucleus to

active gene transcriptions (Packham *et al*., 2015). To date, it is still unclear whether TPX2 and RanBP2 may regulate invasiveness in head and neck cancer. If so, whether MMP-2 and MMP-9 are required for its metastatic function. Understanding the molecular mechanisms of TPX2 and RanBP2 on metastasis in head and neck cancer will be needed for development of more specific biomarkers and novel therapeutic targets.

In conclusion, our study indicated that TPX2 and RanBP2 exhibited increased expression levels in genetically matched pairs of head and neck cancer cell lines. Differential expression patterns at different oral lesions may be due to tumor heterogeneity of HNSCC. How TPX2 and RanBP2 express in such manner needs to be investigated to fully understand their roles in HNSCC pathogenesis.

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REFERENCES

Abe H, Kamai T, Shirataki H, Oyama T, Arai K, Yoshida K (2008). High expression of Ran GTPase is associated with local invasion and metastasis of human clear cell renal cell carcinoma. *Int J Cancer* 122: 2391–2397.

Albert S, Riveiro ME, Halimi C, Hourseau M, Couvelard A, Serova M, Barry B, Raymond E, Faivre S (2013). Focus on the role of the CXCL12/CXCR4 chemokine axis in head and neck squamous cell carcinoma. *Head Neck* 35: 1819–1828.

Argiris A, Karamouzis MV, Raben D, Ferris RL (2008). Head and neck cancer. *Lancet* 371: 1695–1709.

Caceres-Gorriti KY, Carmona E, Barres V, Rahimi K, Letourneau IJ, Tonin PN, Provencher D, Mes-Masson AM (2014). RAN nucleo-cytoplasmic transport and mitotic spindle assembly partners XPO7 and TPX2 are new prognostic biomarkers in serous epithelial ovarian cancer. *PloS One* 9: e91000.

Cardinali M, Pietraszkiewicz H, Ensley JF, Robbins KC (1995). Tyrosine phosphorylation as a marker for aberrantly regulated growth-promoting pathways in cell lines derived from head and neck malignancies. *Int J Cancer* 61: 98–103.

Chang H, Wang J, Tian Y, Xu J, Gou X, Cheng J (2012). The TPX2 gene is a promising diagnostic and therapeutic target for cervical cancer. *Oncol Rep* 27: 1353–1359.

Clarke PR, Zhang C (2001). Ran GTPase: a master regulator of nuclear structure and function during the eukaryotic cell division cycle? *Trends Cell Biol* 11: 366–371.

Dawlaty MM, Malureanu L, Jeganathan KB, Kao E, Sustmann C, Tahk S, Shuai K, Grosschedl R, van Deursen JM (2008). Resolution of sister centromeres requires RanBP2-mediated SUMOylation of topoisomerase IIalpha. *Cell* 133: 103–115.

Deng L, Shang Y, Guo S, Liu C, Zhou L, Sun Y, Nie Y, Fan D, Lu Y, Guo X (2014). Ran GTPase protein promotes metastasis and invasion in pancreatic cancer by deregulating the expression of AR and CXCR4. *Cancer Biol Ther* 15: 1087–1093.

Fan H, Lu Y, Qin H, Zhou Y, Gu Y, Zhou J, Wang X, Fan D (2013). High Ran level is correlated with poor prognosis in patients with colorectal cancer. *Int J Clin Oncol* 18: 856–863.

Felix RS, Colleoni GW, Caballero OL, Yamamoto M, Almeida MS, Andrade VC, Chauffaille Mde L, Silva WA, Jr., Begnami MD, Soares FA, Simpson AJ, Zago MA, Vettore AL (2009). SAGE analysis highlights the importance of p53csv, ddx5, mapkapk2 and ranbp2 to multiple myeloma tumorigenesis. *Cancer Lett* 278: 41–48.

Frohwitter G, Buerger H, Korsching E, van Diest PJ, Kleinheinz J, Fillies T (2017). Site-specific gene expression patterns in oral cancer. *Head Face Med* 13: 6.

Gama-Carvalho M, Carmo-Fonseca M (2001). The rules and roles of nucleocytoplasmic shuttling proteins. *FEBS letters* 498: 157–163.

Hill R, Cautain B, de Pedro N, Link W (2014). Targeting nucleocytoplasmic transport in cancer therapy. *Oncotarget* 5: 11–28.

Iamaroon A, Pattanaporn K, Pongsiriwet S, Wanachantararak S, Prapayasatok S, Jittidecharaks S, Chitapanarux I, Lorvidhaya V (2004). Analysis of 587 cases of oral squamous cell carcinoma in northern Thailand with a focus on young people. *Int J Oral Maxillofac Surg* 33: 84–88.

Kalab P, Heald R (2008). The RanGTP gradient - a GPS for the mitotic spindle. *J Cell Sci* 121: 1577–1586.

Kodama M, Kodama T, Newberg JY, Katayama H, Kobayashi M, Hanash SM, Yoshihara K, Wei Z, Tien JC, Rangel R, Hashimoto K, Mabuchi S, Sawada K, Kimura T, Copeland NG, Jenkins NA (2017). In vivo loss-of-function screens identify KPNB1 as a new druggable oncogene in epithelial ovarian cancer. *Proc. Nat. Acad. Sci. USA* 114: e7301–e7310.

Koontongkaew S, Amornphimoltham P, Monthanpisut P, Saensuk T, Leelakriangsak M (2012). Fibroblasts and extracellular matrix differently modulate MMP activation by primary and metastatic head and neck cancer cells. *Med Oncol* 29: 690–703.

Koontongkaew S, Amornphimoltham P, Yapong B (2009). Tumor-stroma interactions influence cytokine expression and matrix metalloproteinase activities in paired primary and metastatic head and neck cancer cells. *Cell Biol Int* 33: 165–173.

Leemans CR, Braakhuis BJ, Brakenhoff RH (2011). The molecular biology of head and neck cancer. *Nat Rev Cancer* 11: 9–22.

Liu Q, Tu K, Zhang H, Zheng X, Yao Y, Liu Q (2015). TPX2 as a novel prognostic biomarker for hepatocellular carcinoma. *Hepatol Res* 45: 906–918.

Liu Q, Yang P, Tu K, Zhang H, Zheng X, Yao Y, Liu Q (2014). TPX2 knockdown suppressed hepatocellular carcinoma cell invasion via inactivating AKT signaling and inhibiting MMP2 and MMP9 expression. *Chinese J Cancer Res* 26: 410–417.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402–408.

Lu T, Bao Z, Wang Y, Yang L, Lu B, Yan K, Wang S, Wei H, Zhang Z, Cui G (2016). Karyopherinbeta1 regulates proliferation of human glioma cells via Wnt/beta-catenin pathway. *Biochem Biophy Res Commun* 478: 1189–1197.

Ly TK, Wang J, Pereira R, Rojas KS, Peng X, Feng Q, Cerione RA, Wilson KF (2010). Activation of the Ran GTPase is subject to growth factor regulation and can give rise to cellular transformation. *J Biol Chem* 285: 5815–5826.

Martens-de Kemp SR, Nagel R, Stigter-van Walsum M, van der Meulen IH, van Beusechem VW, Braakhuis BJ, Brakenhoff RH (2013). Functional genetic screens identify genes essential for tumor cell survival in head and neck and lung cancer. *Clin Cancer Res* 19: 1994–2003.

Mendez E, Cheng C, Farwell DG, Ricks S, Agoff SN, Futran ND, Weymuller EA, Jr., Maronian NC, Zhao LP, Chen C (2002). Transcriptional expression profiles of oral squamous cell carcinomas. *Cancer* 95: 1482–1494.

Mendez E, Fan W, Choi P, Agoff SN, Whipple M, Farwell DG, Futran ND, Weymuller EA, Jr., Zhao LP, Chen C (2007). Tumor-specific genetic expression profile of metastatic oral squamous cell carcinoma. *Head Neck* 29: 803–814.

Packham S, Warsito D, Lin Y, Sadi S, Karlsson R, Sehat B, Larsson O (2015). Nuclear translocation of IGF-1R via p150(Glued) and an importin-beta/RanBP2-dependent pathway in cancer cells. *Oncogene* 34: 2227–2238.

Perez-Ordonez B, Beauchemin M, Jordan RC (2006). Molecular biology of squamous cell carcinoma of the head and neck. *Journal of clinical pathology* 59: 445–453.

Rensen WM, Mangiacasale R, Ciciarello M, Lavia P (2008). The GTPase Ran: regulation of cell life and potential roles in cell transformation. *Front Biosci* 13: 4097–4121.

Roepman P, Wessels LF, Kettelarij N, Kemmeren P, Miles AJ, Lijnzaad P, Tilanus MG, Koole R, Hordijk GJ, van der Vliet PC, Reinders MJ, Slootweg PJ, Holstege FC (2005). An expression profile for diagnosis of lymph node metastases from primary head and neck squamous cell carcinomas. *Nat Genet* 37: 182–186.

Rosenthal EL, Matrisian LM (2006). Matrix metalloproteases in head and neck cancer. *Head Neck* 28: 639–648.

Severino P, Alvares AM, Michaluart P, Jr., Okamoto OK, Nunes FD, Moreira-Filho CA, Tajara EH (2008). Global gene expression profiling of oral cavity cancers suggests molecular heterogeneity within anatomic subsites. *BMC Res Notes* 1: 113.

Squire JA, Bayani J, Luk C, Unwin L, Tokunaga J, MacMillan C, Irish J, Brown D, Gullane P, Kamel-Reid S (2002). Molecular cytogenetic analysis of head and neck squamous cell carcinoma: By comparative genomic hybridization, spectral karyotyping, and expression array analysis. *Head Neck* 24: 874–887.

Stadler ME, Patel MR, Couch ME, Hayes DN (2008). Molecular biology of head and neck cancer: risks and pathways. *Hematol Oncol Clin North Am* 22: 1099–1124.

van der Watt PJ, Maske CP, Hendricks DT, Parker MI, Denny L, Govender D, Birrer MJ, Leaner VD (2009). The Karyopherin proteins, Crm1 and Karyopherin beta1, are overexpressed in cervical cancer and are critical for cancer cell survival and proliferation. *Int J Cancer* 124: 1829-1840.

Vogel C, Marcotte EM (2012). Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat Rev Genet* 13: 227–232.

Warner SL, Stephens BJ, Nwokenkwo S, Hostetter G, Sugeng A, Hidalgo M, Trent JM, Han H, Von Hoff DD (2009). Validation of TPX2 as a potential therapeutic target in pancreatic cancer cells. *Clin Cancer Res* 15: 6519–6528.

Wei P, Zhang N, Xu Y, Li X, Shi D, Wang Y, Li D, Cai S (2013). TPX2 is a novel prognostic marker for the growth and metastasis of colon cancer. *J Transl Med* 11: 313.

Xia F, Canovas PM, Guadagno TM, Altieri DC (2008a). A survivin-ran complex regulates spindle formation in tumor cells. *Mol Cell Biol* 28: 5299–5311.

Xia F, Lee CW, Altieri DC (2008b). Tumor cell dependence on Ran-GTP-directed mitosis. *Cancer Res* 68: 1826–1833.

Yang L, Hu B, Zhang Y, Qiang S, Cai J, Huang W, Gong C, Zhang T, Zhang S, Xu P, Wu X, Liu J (2015). Suppression of the nuclear transporter-KPNbeta1 expression inhibits tumor proliferation in hepatocellular carcinoma. *Med Oncol* 32: 128.

Yu T, Wu Y, Helman JI, Wen Y, Wang C, Li L (2011). CXCR4 promotes oral squamous cell carcinoma migration and invasion through inducing expression of MMP-9 and MMP-13 via the ERK signaling pathway. *Mol Cancer Res* 9: 161–172.

Ziober AF, Patel KR, Alawi F, Gimotty P, Weber RS, Feldman MM, Chalian AA, Weinstein GS, Hunt J, Ziober BL (2006). Identification of a gene signature for rapid screening of oral squamous cell carcinoma. *Clin Cancer Res* 12: 5960–5971.