

## ABSTRACT

Nasopharyngeal carcinoma (NPC) is a highly metastatic cancer that is endemic in South East Asia and Southern China. Despite the gravity of the disease, the current knowledge on its molecular pathogenesis is still inadequate to improve the disease management. The present study seeks to understand the molecular mechanism of NPC, with an aim to identify potential therapeutic targets or biomarkers. From a previous expression microarray study, Four – Jointed Box 1 (FJX1) gene was found to be upregulated in NPC compared to non-cancerous controls with negligible expression in 5 vital normal human organs. Human FJX1 is a *Drosophila* orthologue of *four-jointed (fj)* gene which codes for a Golgi-resident kinase that phosphorylates specific cadherin domains and functions downstream of the Notch and Hippo signaling pathways. The overexpression of FJX1 in primary NPC tissues was confirmed at both mRNA and protein levels, while its low expression was validated in 16 normal human organs. Both overexpression and knockdown experiments showed that FJX1 increased the aggressiveness of NPC cells by promoting cell proliferation, invasion and anchorage-independent growth. Concomitant change of Cyclins D1 and E1 levels were observed with FJX1 level, suggesting FJX1 enhances cell proliferation through cell cycle regulation. The results of the present study demonstrate for the first time the overexpression of FJX1 in NPC as a putative oncogene, and it represents an attractive therapeutic target for NPC.

## ABSTRAK

Karsinoma nasofaring (KNF), endemik di Asia Tenggara dan Selatan China, merupakan sejenis kanser yang sangat mudah bermetastasis. Walaupun penyakit ini membawa kesan yang teruk kepada penghidapnya, pengetahuan mengenai pertumbuhan penyakit ini di peringkat molekul masih lagi tidak mencukupi untuk memperbaiki cara pengurusan penyakit tersebut. Kajian ini bertujuan untuk memahami mekanisma molekular KNF, dengan tujuan untuk mengenal pasti gen – gen yang boleh dijadikan sebagai bakal gen sasaran dalam rawatan atau sebagai gen penanda. Dengan berpandukan kajian mikroatur pengekspresan yang terdahulu, ekspresi gen Four-Jointed Box 1 (FJX1) telah dikenal pasti berada di tahap yang tinggi di KNF berbanding kumpulan kawalan bukan kanser. Ekspresi FJX1 di 5 organ manusia penting yang normal pula berada pada tahap yang sangat rendah. Gen manusia FJX1 adalah ortolog kepada gen *Drosophila* bergelar *four-jointed (fj)*, yang mana protinnya merupakan kinase di Golgi yang memfosforilasi domain-domain cadherin tertentu. *Fj* juga berfungsi di bawah kawalan tapak jalan Notch dan Hippo. Peningkatan FJX1 di KNF telah dipastikan pada kedua-dua tahap, baik di tahap mRNA mahupun di tahap protin. Ekspresinya juga telah disahkan rendah di 16 organ manusia normal. Eksperimen–eksperimen *in vitro* telah menunjukkan bahawa FJX1 meningkatkan sifat agresif sel-sel KNF dengan menggalakkan pertubuhan, penyerbuan, dan pertumbuhan tanpa lekap sel-sel tersebut. Perubahan sekali gus tahap Cyclin D1 dan Cyclin E1 bersama-sama tahap FJX1 juga didapati — ini menunjukkan bahawa FJX1 menggalakkan pertumbuhan melalui proses kitaran sel. Hasil kajian ini membuktikan buat julung kalinya peningkatan tahap FJX1 di KNF, dan sebagai sebagai bakal gen penyebab kanser, gen ini juga merupakan sasaran bagi rawatan yang berpotensi bagi KNF.

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Nothing is permanent in this wicked world – not even our troubles.

Charlie Chaplin.

Actor, director, screenwriter (1889 – 1977)

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## LIST OF SYMBOLS AND ABBREVIATIONS

|                 |                       |
|-----------------|-----------------------|
| °C              | degrees Celcius       |
| μg              | microgram             |
| μl              | microliter            |
| μM              | micromolar            |
| bp              | base pair             |
| <i>g</i>        | force of gravity      |
| g               | gram                  |
| hr              | hour                  |
| kb              | kilobase pair         |
| kDa             | kiloDalton            |
| M               | molar                 |
| mg              | milligram             |
| min             | minute (time)         |
| ml              | milliliter            |
| mM              | millimolar            |
| mm <sup>2</sup> | milimeter square      |
| ng              | nanogram              |
| rpm             | revolution per minute |
| sec             | second (time)         |
| U               | enzyme unit           |
| v/v             | volume per volume     |

|               |   |
|---------------|---|
| ADPRT         | poly (ADP-ribose) polymerase 1  |
| BART          | <i>Bam</i> HIA rightward transcripts  |
| BCL-2         | B-cell leukemia/lymphoma 2  |
| BLAST         | Basic Local Alignment Search Tool   |
| BSA           | bovine serum albumin  |
| CapG          | capping protein (actin filament), gelsolin-like                               |
| CARIF         | Cancer Research Initiatives Foundation  |
| CCND1         | cyclin D1   |
| CCNE1         | cyclin E1   |
| CD200         | cluster of differentiation 200  |
| CD44          | cluster of differentiation 44   |
| CDK2          | cyclin dependent kinase 2   |
| CDK4          | cyclin dependent kinase 4   |
| CDKN1B        | cyclin-dependent kinase inhibitor 1B  |
| CDKN2A-CDKN2B | cyclin-dependent kinase inhibitor 2A-cyclin-dependent kinase 4<br>inhibitor B |
| cDNA          | complementary DNA   |
| CGH           | comparative genomic hybridization   |
| CHEK1         | CHK1 checkpoint homolog ( <i>S. pombe</i> )                                   |
| CK10          | keratin 10  |
| CK5           | keratin 5   |
| CLCA2         | chloride channel accessory 2  |

|                 |  |
|-----------------|--|
| CLDN1           | claudin 1  |
| CLIC1           | chloride intracellular channel 1   |
| CO <sub>2</sub> | carbon dioxide   |
| CT              | cycle threshold  |
| DAB             | diamino benzidine  |
| DMSO            | dimethyl sulfoxide   |
| DNA             | deoxyribonucleic acid  |
| DNA-PKC         | deoxyribonucleic acid-protein kinase catalytic polypeptide                               |
| dNTP            | deoxynucleotide triphosphate   |
| DPX             | <i>P</i> -xylylene- <i>A,A'</i> -bispyridinium dibromide                                 |
| Ds              | dachsous   |
| DTT             | dithiothreitol   |
| EBER1           | Epstein-Barr virus encoded-RNA 1   |
| EBER2           | Epstein-Barr virus encoded-RNA 2   |
| EBNA1           | Epstein-Barr nuclear antigen   |
| EBV             | Epstein-Barr virus   |
| EDTA            | ethylenediaminetetraacetic acid  |
| EGF             | epidermal growth factor  |
| EGFR            | epidermal growth factor receptor   |
| EHS             | Engelbreth-Holm-Swarm  |
| EM              | extracellular matrix   |
| EMT             | epithelial-to-mesenchymal transition   |
| ERCC1           | excision repair cross-complementing rodent repair deficiency,<br>complementation group 1 |

|              |  |
|--------------|--|
| Erk          | mitogen-activated protein kinase 1                   |
| ESI-Q-TOF MS | electrospray ionization-quadrupole time-of-flight MS |
| EST          | expressed sequence tag                               |
| EZH2         | enhancer of zeste homolog 2 (Drosophila)             |
| FACS         | fluorescent activated cell sorter                    |
| Fat4         | FAT tumor suppressor homolog 4 (Drosophila)          |
| FBS          | fetal bovine serum                                   |
| FFPE         | formalin-fixed paraffin embedded                     |
| FGFR3        | fibroblast growth factor receptor 3                  |
| Fj           | four-jointed   |
| FJX1         | four-jointed box 1                                   |
| Ft           | fat  |
| FZD6         | frizzled family receptor 6                           |
| FZD7         | frizzled family receptor 7                           |
| GABBR1       | gamma-aminobutyric acid (GABA) B receptor, 1         |
| GAPDH        | glyceraldehyde-3-phosphate dehydrogenase             |
| GSK-3beta    | glycogen synthase kinase 3 beta                      |
| HCl          | hydrochloric acid                                    |
| HDAC1        | histone deacetylase 1                                |
| HLA          | Human Leukocyte Antigen                              |
| HMGB1        | high-mobility group box 1                            |
| HOGG1        | 8-oxoguanine DNA glycosylase                         |
| IMRT         | intensity-modulated radiotherapy                     |
| IPTG         | isopropyl $\beta$ -D-1-thiogalactopyranoside         |



|                                 |   |
|---------------------------------|---|
| ITGA9                           | integrin alpha-9  |
| JAK/STAT                        | janus kinase / signal transducer and activator of transcription |
| KSFM                            | keratinocyte serum-free medium                                  |
| LATS2                           | large tumor suppressor, homolog 2 (Drosophila)                  |
| LB                              | Laura-Bertani   |
| LMP2A                           | latent membrane protein 2A                                      |
| LMP2B                           | latent membrane protein 2B                                      |
| MDS1-EVI1                       | ecotropic viral integration site 1                              |
| Mek                             | mitogen-activated protein kinase kinase 1                       |
| MgCl <sub>2</sub>               | magnesium chloride  |
| MHC                             | Major Histocompatibility Complex                                |
| miRNA                           | micro RNA   |
| mRNA                            | messenger RNA   |
| MS                              | mass spectrometry   |
| MTC                             | Multiple Tissue cDNA  |
| Myc                             | myelocytomatosis viral oncogene homolog (avian)                 |
| Na <sub>3</sub> VO <sub>4</sub> | Sodium orthovanadate  |
| NaCl                            | Sodium chloride   |
| NaOH                            | Sodium hydroxide  |
| NP-40                           | nonyl phenoxyethoxyethanol                                      |
| NPC                             | nasopharyngeal carcinoma  |
| P53                             | protein 53  |
| PAGE                            | polyacrylamide gel  |
| PBL                             | peripheral blood leukocyte                                      |

|         |  |
|---------|--|
| PBS     | phosphate buffer saline                                      |
| PBST    | phosphate buffer saline Tween-20                             |
| PCR     | polymerase chain reaction                                    |
| pEGFR   | phosphorylated EGFR  |
| pERK    | phosphorylated ERK   |
| PRKDC   | protein kinase, DNA-activated, catalytic polypeptide         |
| qPCR    | quantitative real-time PCR                                   |
| RAD23A  | UV excision repair protein RAD23 homolog A                   |
| RAD23B  | UV excision repair protein RAD23 homolog B                   |
| Raf     | v-raf murine leukemia viral oncogene homolog                 |
| RALA    | v-ral simian leukemia viral oncogene homolog A (ras related) |
| Ras     | RAS p21 protein activator (GTPase activating protein) 1      |
| RASSF1A | ras association domain-containing protein 1A                 |
| RASSF2  | ras association domain-containing protein 2                  |
| RIPA    | radioimmuno precipitation assay                              |
| RKIP    | phosphatidylethanolamine binding protein 1                   |
| RNA     | ribonucleic acid   |
| RNAi    | RNA interference   |
| RPMI    | Roswell Park Memorial Institute medium                       |
| RQ      | relative quantification                                      |
| S100A9  | S100 calcium binding protein A9                              |
| SCCA1   | serpin peptidase inhibitor, clade B (ovalbumin), member 4    |
| SDS     | sodium dodecyl sulphate                                      |

|              |   |
|--------------|---|
| SELDI-TOF MS | surface-enhanced laser desorption/ionization time-of-flight mass spectrometry         |
| semi-qPCR    | semi-quantitative PCR   |
| siRNA        | small interfering RNA   |
| STR          | single tandem repeat  |
| TNFRSF19     | tumor necrosis factor receptor superfamily, member 19                                 |
| Tris         | tris (hydroxymethyl) aminomethane   |
| USA          | the United States of America  |
| UTR          | untranslated region   |
| UV           | ultra violet  |
| VEGF         | vascular endothelial growth factor  |
| WHO          | World Health Organization   |
| WIF-1        | wnt inhibitory factor 1   |
| WNT5A        | wingless-type MMTV integration site family, member 5A                                 |
| X-Gal        | bromo-chloro-indolyl-galactopyranoside  |
| XPC          | xeroderma pigmentosum, complementation group C  |
| XPD          | excision repair cross-complementing rodent repair deficiency, complementation group 2 |
| XRCC1        | X-ray repair complementing defective repair in Chinese hamster cells 1                |
| YAP          | yes-associated protein 1  |
| ZO-1         | tight junction protein 1 (zona occludens 1)   |