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**Biological properties of tumour subpopulations in nasopharyngeal carcinoma**

1,2Susan Ling Ling Hoe, 1Lu Ping Tan, 1Norazlin Abdul Aziz, 1Kitson Liew, 3Sin-Yeang Teow, 1Fazlyn Reeny Abdul Razak, 2Yoon Ming Chin, 1Nurul Ashikin Mohamed Shahrehan, 1Tai Lin Chu, 4Noor Kaslina Mohd Kornain, 3Suat-Cheng Peh 5Cheng Eng Koay, 1Munirah Ahmad, 2Ching-Ching Ng, 1Alan Soo-Beng Khoo\*

*1 Molecular Pathology Unit, Cancer Research Centre, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia.2 Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia.3 Sunway Institute for Healthcare Development, Sunway University, 47500 Bandar Sunway, Selangor, Malaysia.4 Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA (UiTM), 47000 Sungai Buloh, Selangor, Malaysia.5 Gleneagles Hospital (Kuala Lumpur) Sdn. Bhd., Jalan Ampang, 50450 Kuala Lumpur, Malaysia*

**ABSTRACT**

Nasopharyngeal carcinoma (NPC) is the most common type of nasopharyngeal tumour affecting people of Southern Chinese descent, the Bidayuhs of Sarawak, North Africans from Tunisia and Algeria, and the Inuits of Alaska, Greenland and North Canada. It is the second most common cancer in Malaysian males of working age of 25 to 59 years, with most patients detected at Stages III and IV. Studies on other solid malignancies have recognised the presence and functions of tumour subpopulations particularly in drug resistance, metastasis and recurrence. The objectives of our study are to evaluate the biological properties of such subpopulations identified by CD24, CD44 and EpCAM surface markers in a NPC patient-derived xenograft (PDX) and to determine the relevance of our findings in NPC patient specimens. Briefly, cell sorting technique was used to isolate NPC subpopulations from the PDX, followed by in vivo tumourigenicity, serial-transplantation assay to test for self-renewal ability, cell cycle, differential gene expression, gene modulation and immunofluorescence staining analyses. Finally, RNA sequencing data was mined from a separate study for EPCAM and other selected transcript levels. Bright expressing cell subpopulations isolated with CD44 and EpCAM markers (“CD44br” and “EpCAMbr”) in NPC PDX contained higher S-phase content and faster-growing tumourigenic cells which resulted in larger tumours with increased mitotic figures, compared to the respective dim subset. Cell subpopulations isolated by the three markers were able to self-renew for at least four generations. CD44br and EpCAMbr cells had lower KLF4 and p21 mRNA and protein expressions. KLF4 overexpression in EpCAMbr cells led to growth reduction, whilst chemical inhibition of KLF4 induced in vitro cell growth. EPCAM transcript level was higher in NPC patient specimens than in non-NPC patients. It was also incrementally increased according to disease stage. In conclusion, EpCAM is potentially a poor prognosis marker for NPC.