Dynamic network biomarker indicates pulmonary metastasis at the tipping point of hepatocellular carcinoma

With the support by the National Natural Science Foundation of China, the research team directed by Prof. Xia JingLin (夏景林) at the Liver Cancer Institute, Zhongshan Hospital, Fudan University, and Prof. Chen LuoNan at Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, recently reported that CALML3 as a dynamic network biomarker indicated pulmonary metastasis at the tipping point of hepatocellular carcinoma, which was published in *Nature Communications* (2018, 9: 678).

One major cause of high mortality of HCC is its high rate of metastasis. Thus, it is important to explore predictive biomarkers of the pre-metastatic state and molecular pathology of metastasis initiation for early diagnosis or prevention. They developed a prediction model of pulmonary metastasis based on dynamic network biomarkers (DNBs), which showed superiority in identifying the critical or pre-metastatic state (a tipping point just before the dramatic transition to a metastatic state) during disease progression via interactions between molecules (differential networks) in a dynamic manner. By analyzing the corresponding functional network of DNB and time-series transcriptomic data, they identified the tipping point of metastasis initiation (the third week after orthotopic implantation in a mouse model), which was consistent with circulating tumour cells analysis. Furthermore, they found that calmodulin-like-protein (CALML3), a calcium sensor protein, was one of DNB members and played an important role in metastasis initiation.

Loss of CALML3 was closely correlated with poor prognosis of HCC patients as showed by survival analysis. To further elucidate the role of CALML3 in HCC metastasis, their group performed gain-of-function and loss-of-function assays *in vitro* and *in vivo*. They demonstrated that CALML3 could significantly inhibit HCC carcinogenesis and metastasis initiation. In addition, they revealed the functional role of CALML3 at a network level in the initiation of metastasis. According to a knowledge-based molecular network, they identified the genes and biological functions that were directly affected by CALML3 during metastasis initiation. Moreover, by PCR array detection they found that typical metastasis-related genes were regulated by CALML3 in a cascade. These results demonstrated that CALML3, as a tumour suppressor, played an important role in protecting against pulmonary metastasis by regulating multiple signalling pathways.

These findings may provide new insight into identifying the tipping point of HCC pulmonary metastasis and molecular pathology of metastasis from the perspectives of dynamics and network. They also proposed CALML3 as an early-warning indicator of the initiation of metastasis.

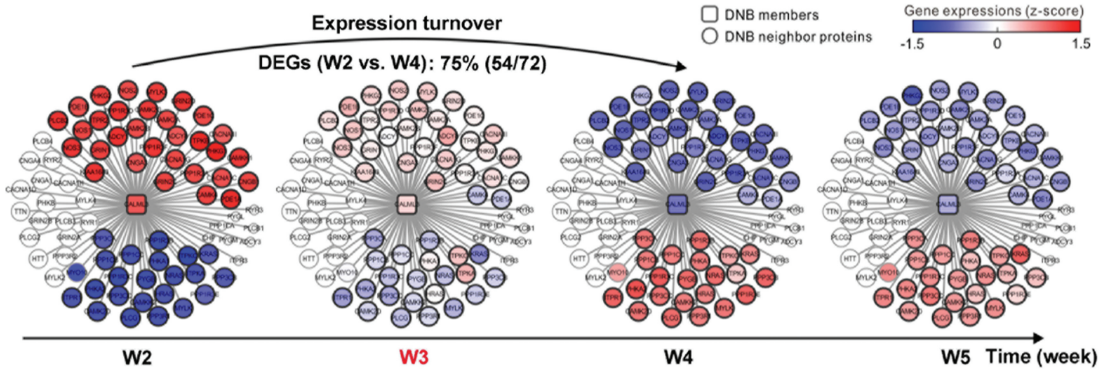

Figure The Dynamics of CALML3 and its affected genes in terms of expression and function during the progression of pulmonary metastasis.