c-MYC depletion potentiates cisplatin-induced apoptosis in head and neck squamous cell carcinoma: involvement of TSP-1 up-regulation

c-MYC, the human homolog of a retroviral oncogene, was identified 25 years ago. Given the crucial role of c-MYC in cell proliferation and death and, moreover, in the development and maintenance of many cancers, the potential therapeutic benefits of c-MYC inhibition in human malignancies are self-evident. However, since c-MYC is deregulated and overexpressed in such a broad range of human cancers and it regulates approximately 10%–15% of all cellular genes, it is difficult to determine what kinds of tumor are the appropriate candidates for this anticancer strategy [1, 2].

Recent investigations highlight the tumorigenic role of c-MYC in head and neck squamous cell carcinoma (HNSCC). Koehn et al. applied proteomic technologies to analyze the proteome of 10 patients with human oral squamous cell carcinoma and reported that, among 350 different gene products identified, 20 proteins showed deranged levels in this disease and are potentially involved in tumor growth and metastasis. By pathway analysis, the authors found 8 of the 16 up-regulated gene products to be linked to three main locus genes, one of which is c-MYC [3]. Moreover, cancerous inhibitor of protein phosphatase 2A (CIP2A) a novel oncoprotein, was first identified to be required for the development of human malignancies in HNSCC model. Importantly, CIP2A plays its oncogenic role mainly through stabilizing c-MYC protein [4]. All these information provide the rationale to evaluate the therapeutic potential of targeting aspects of c-MYC activity in HNSCC.

Thrombospondin-1 (TSP-1) was recently identified as a target gene of c-MYC [5]. It is a multimodular, 420-kDa,
homotrimeric, matricellular glycoprotein that regulates cell proliferation, migration and cell death in a variety of physiological and pathological settings, including wound healing, inflammation, angiogenesis and neoplasia. TSP-1 has been described to induce apoptosis through caspase-dependent and -independent mechanisms. For HNSCC, TSP-1 was found to be down-regulated in patient samples using high-throughput protein microarray techniques [6].

Our preliminary evidence indicated that c-MYC depletion potentiates cisplatin-induced apoptosis in HNSCC at least partly through up-regulation of TSP-1. Stable c-MYC gene silencing by short hairpin RNA (shRNA) approach resulted in a marked up-regulation of endogenous TSP-1 expression and a significant increase in generation of apoptosis following cisplatin treatment in a commonly used HNSCC cell line, SCC-25 (Figure 1A and B). In the present study, we used two shRNA-expressing plasmids with different target sequences (shMYC-1/-2) to exclude off-target effects. Furthermore, as shown in Figure 1C, when we resuppressed TSP-1 expression by small interfering RNA (siRNA) technique in the c-MYC-down-regulated SCC-25 cells, the sensitization of cells to cisplatin-induced apoptosis was markedly abolished. We used stable transfectants expressing shMYC-2 to carry out this assay due to its higher efficacy of c-MYC knockdown than the other transfectant shMYC-1 in SCC-25 cells, as determined in Figure 1A. TSP-1 induction by c-MYC knockdown and resuppression by siRNA approach in experiments described in panel (C) were confirmed by western blot analysis. *P < 0.05, **P < 0.01.

Collectively, our data presented here strongly indicate a therapeutic potential of targeting c-MYC–TSP-1 axis in HNSCC. It would be of great interest to further investigate the role of c-MYC–TSP-1 pathway in the pathogenesis of HNSCC and other human malignancies.

B. Xu¹, P. Liu²*, J. Li² & H. Lu²
Departments of ¹Internal Medicine, ²Hematology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, People’s Republic of China
(*E-mail: liupeng8888@yahoo.com.cn)

funding
National Natural Science Foundation of China (30500603); Ministry of Science and Technology of China ‘863’ Project (2003AA205060); Chuangxin Foundation of Nanjing Medical University (CX2003004).
disclosure
No conflicts of interest to declare.

references

doi:10.1093/annonc/mdp567
Published online 15 December 2009