Medical Sciences

Prevention of Hepatocellular carcinoma progression by the Chinese herb derived peptide Lingzhi-8

Chao-Chuan Wu*[,%], Chi-Tan Hu[#,%], Ming-Che Lee[※], Yao-Jen Chang*, Siou-Mei Pan[#], Jia-Ru Wu[&], Shu-Chuan Lin[#], Wen-Sheng Wu[&,1]

*Department of Surgery, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation. School of Medicine, Tzu Chi University. [#]Research Centre for Hepatology, Department of Internal Medicine, Buddhist Tzu Chi General Hospital. School of Medicine, Tzu Chi University, Hualien, Taiwan. [※]Department of Surgery, Buddhist Tzu Chi General Hospital, Hualien. School of Medicine, Tzu Chi University. [&]Institute of Medical Sciences, Department of Laboratory Medicine and Biotechnology, College of Medicine, Tzu Chi University, Hualien, Taiwan

Hepatocellular carcinoma (HCC) ranks sixth in incidence and third in mortality among all cancers worldwide. Metastatic spreads are responsible for poor prognosis of most HCC. The receptor tyrosine kinase of hepatocyte growth factor (HGF), c-Met, is now regarded as one of the promising therapeutic targets for prevention of HCC progression. However, c-Met is positive in only 20% to 48% of human HCC cases. On the other hand, the side effects caused by conventional c-Met inhibitors were frequently observed. It is urgent to search more efficient antagonists with safety warrant for prevention of HCC progression. One promising anti-HCC agent is the medicinal peptide LZ-8 (also known as Lingzhi-8), which is purified from the Chinese herbal drug Ganoderma lucidium. Previously LZ-8 has been found to acquire both immunomodulatory and anticancer capability. Recently, LZ-8 was found to be an effective antagonist for prevention the progression of a lot of patient derived HCC cell lines both in vitro and in vivo. LZ-8 may block c-Met-dependent or c-Met-independent signaling to achieve its anti-HCC effects. Deregulated endosomal signaling of c-Met is recently highlighted to be responsible for c-Met dependent tumor progression. Being a peptide with Lectin like activity, LZ-8 may disturb c-Met endosomal processing by promoting the lysosomal degradation of c-Met, resulting in decreased c-Met signaling. In the future, LZ-8 may be proved as a more efficient and safe anti-HCC agent than the conventional drugs. *Journal of Nature and Science, 1(3):e50, 2015.*

Hepatocellular carcinoma | Chinese herb | Lingzhi-8 | LZ-8 | c-Met

Targeting HGF-c-Met signaling for progression of Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) ranks sixth in incidence and third in mortality among all cancers worldwide [1]. Recently, sorafenib, one of the multi-kinase inhibitors with anti-proliferative, and pro-apoptotic properties, was found to be the most promising agent for HCC management [2-4]. However, the overall outcomes are far from satisfactory with improvement of overall survival for less than one year [5]. Moreover, the acquired resistance and side effect to sorafenib has drawn attention [6]. Since metastatic spreads are responsible for poor prognosis of most HCC [7, 8], it is not surprising to find the limited response to anti-proliferative drugs such as sorafenib. Thus far, effective target therapy aiming at the molecular pathway leading to tumor metastasis of HCC has not been well established.

Tumor metastasis occurs via complicated processes, including epithelial mesenchymal transition (EMT), migration and invasion of primary tumor, followed by intravasation, extravasation and colonization at the metastatic loci [9]. Within tumor microenvironment the primary tumor may interact with stromal and inflammatory cells leading to the secretion of a lot of metastatic factors including hepatocyte growth factor (HGF) [9-12], epidermal growth factor (EGF), and transforming growth factor-β (TGFβ) [13]. Among the metastatic factors HGF, a well-known scatter factor, was highlighted in the progression of cancer [14] including HCC. The receptor tyrosine kinase (RTK) of HGF, c-Met, is deregulated in HCCs which were closely associated with early HCC recurrence [16]. Patients with high c-Met expressing HCCs usually have shorter 5-year survival after curative resection [15-19]. On the other hand, the effects of HGF on metastatic changes of HCC including EMT, migration and invasion have been established [20-22]. Therefore, HGF-c-Met signaling is now regarded as one of the promising therapeutic targets for prevention of HCC progression [2, 23-25].

Up to now, at least 17 c-Met inhibitors including JNJ-38877605, GEN-203, and ARQ197 are under clinical evaluation [26]. However, early clinical trials have revealed resistance to c-Met inhibition [24], probably due to negative c-Met signaling in these HCC (c-Met is positive in only 20% to 48% of human HCC cases [17]). On the other hand, the side effects caused by c-Met inhibitors including anemia, neutropenia and liver and bone marrow toxicity [27, 28] were frequently observed. Taken together, it is urgent to search more efficient antagonists with safety warrant which is capable of blocking critical molecular pathway, either c-Met dependent or independent, for prevention of HCC progression.

This review focuses on the preclinical trials regarding the anti-HCC effect of Chinese herbal derived medicinal peptide LZ-8. The proposed mechanisms for LZ-8 to suppress c-Met signaling are also discussed.

Preventive effect of Lingzhi-8 on tumor progression of hepatocellular carcinoma

Currently, many potential Chinese herb-derived anticancer compounds have been explored. For example, Celastrol, extracted from the root of Tripterygiumwilfordii Hook, was found to have anti–prostate cancer properties [29]. Moreover, Platycodin D (PD) [30] and TDP [31], isolated from the traditional Chinese herb, Platycodonis radix and Garcinia oblongifolia, respectively, have anticancer potential in HCC. One promising anti-HCC agent is the medicinal peptide LZ-8 (also known as Lingzhi-8), which is purified from the Chinese herbal drug Ganoderma lucidium [32]. In previous studies, LZ-8 was found to be an immunomodulatory adjuvant that enhanced the efficacy of cancer DNA vaccines by activating dendritic cells [33, 34]. More recently, the prominent anticancer capability of LZ-8 was highlighted. LZ-8 may suppress proliferation of breast cancer [35] and lung cancer [36, 37] and retard cell migration of cervical cancer [38]. LZ-8 may induces endoplasmic reticulum (ER) stress to trigger over-autophagy response leading to cell death of the human gastric cancer cell line SGC-7901 [39]. The molecular mechanisms for the antitumor activity of LZ-8 have been intensively studied. For example, LZ-8 may stabilize p53 and increase the CDK inhibitor $p21^{cip}$ [40], leading to cell cycle arrest of lung cancer cells. In addition, LZ-8 can repress telomerase activity [41] in lung adenocarcinoma cells. In regard with intracellular signaling, LZ-8 may suppress the protein kinase C-dependent pathway [42], involved in cancer progression triggered by HGF-c-Met signaling [43].

In our recent report [44], we found LZ-8 may serve as an effective antagonist for prevention of tumor progression of a lot of

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[1]Corresponding Author: Wen-Sheng Wu. Institute of medical biotechnology, College of Medicine, Tzu Chi University No. 701, Chung Yang Rd, Sec 3, Hualien 970, Taiwan. Phone: 8867-03-8565301 ext 2327；Fax: 8867-03-8571917. Email: wuwstcu1234@yahoo.com.tw.

[%] Chao-Chuan Wu and Chi-Tan Hu contribute equally to the paper

patient derived HCC cell lines. These cell lines have been characterized for the status of c-Met signaling. As demonstrated in the report, cell migration of both c-Met positive and c-Met negative HCC, HCC372 and HCC329, respectively, can be suppressed by LZ-8. In the animal experiment, LZ-8 suppressed intrahepatic metastasis of HCC329 [44] and another c-Met positive HCC, HCC340 (unpublished result), in SCID mice.

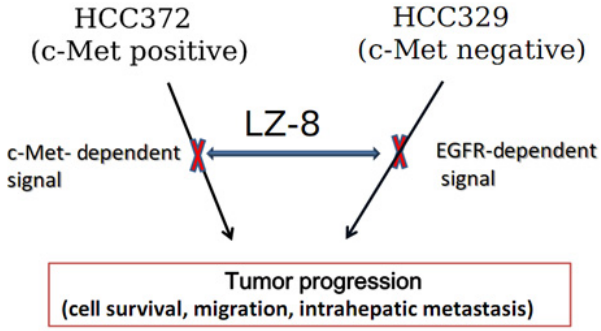

Fig.1 LZ-8 suppress tumor progression of HCC via blockade of c-Met dependent or in dependent signaling. LZ-8 may block c-Met-dependent or EGFR-dependent signaling for suppressing the progression of c-Met-positive and negative HCCs.

The anti-HCC effects of LZ-8 on HCC progression were found to be closely associated with the blockade of critical signal transduction [44]. For the c-Met positive HCC372, LZ-8 suppressed both the expression of c-Met and phosphorylation of c-Met (at Tyr1234), which accompanied the decrease of the downstream ERK signaling and the inhibition of cell migration. On the other hand, to identify the signaling pathway mediating cell migration of the c-Met-negative HCC329, receptor array was used to screen the activities of RTKs that are deregulated in this cell. Among the 49 phosphorylated RTKs (p-RTKs) examined, p-EGFR was found to be the most highly upregulated in HCC329. Importantly, treatment of HCC329 with LZ-8 for 16-24 h specifically decreased p-EGFR by 70%. Consistently, the EGFR inhibitor AG1748 suppress cell migration of HCC329 as effective as LZ-8. In addition, LZ-8 may significantly decreased cell survival of both HCC372 and HCC 329. Taken together, LZ-8 may block c-Met-dependent or c-Met-independent signaling for anti-HCC progression (summarized in Fig.1).

Proposed mechanisms for LZ-8 to block c-Met signaling: implication of the c-Met endocytosis

The underlying mechanisms for LZ-8 to block c-Met signaling are being investigated. Since LZ-8 may decrease both expression and phosphorylation of c-Met, it is probable that LZ-8 can affect the gene transcription of c-Met. However, this was excluded by our recent experiments demonstrating that LZ-8 didn't decrease the c-Met mRNA level in HCC372 (unpublished result). Therefore, LZ-8 may influence the stability of c-Met on the posttranslational level. Recently, the HGF-triggered c-Met endosomal signaling [for review 45] is highlighted in tumor progression [46]. Increased endocytosis/recycling of c-Met coupled with decreased lysosomal degradation of c-Met were responsible for enhanced activation of the GTPase Rac1, leading to increased cell migration, invasion and metastasis of breast cancer cells [46]. Therefore, modifications in the balance between degradation and recycling of c-Met may be a promising strategy for blocking c-Met signaling. We have found LZ-8 and Con A, a mannose specific Lectin, may prevent HGF-induced tumor progression of the HepG2 via blockade of c-Met endosomal signaling (unpublished result). Interestingly, the major biological activities of LZ-8 resemble the mitogenic Lectin which is capable of inducing cellular aggregate formation [47, 48]. Therefore, it is tempting to speculate that LZ-8 may disturb c-Met endosomal processing in HCC372 by promoting the lysosomal degradation of c-Met and decreased c-Met signaling.

Conclusion and perspective

LZ-8 acquires broad capabilities of suppressing the critical signal pathways, either c-Met- dependent or independent, mediating the progression of diverse HCCs. Being an active component of the Chinese herbal Lingzhi, the safety concern of LZ-8 has been approved. Whether LZ-8 can be a more efficient anti-HCC agent than the conventional drugs is worthy of further clinical investigation.

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, et al. (2008) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127: 2893-2917.
2. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, et al. (2008) Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359: 378-390.
3. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, et al. (2009) Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 10: 25-34.
4. Llovet JM, Ducreux M, Lencioni R, Di Bisceglie AM, Galle PR, et al. (2012) EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 56: 908-943.
5. Zhu AX (2012) Molecularly targeted therapy for advanced hepatocellular carcinoma in 2012: current status and future perspectives. Semin Oncol 39: 493-502.
6. Zhai B, Sun XY (2013) Mechanisms of resistance to sorafenib and the corresponding strategies in hepatocellular carcinoma. World J Hepatol 5: 345-352.
7. Bosch FX, Ribes J, Diaz M, Cleries R (2004) Primary liver cancer: worldwide incidence and trends. Gastroenterology 127: S5-S16.
8. Tang ZY, Ye SL, Liu YK, Qin LX, Sun HC, et al. (2004) A decade's studies on metastasis of hepatocellular carcinoma. J Cancer Res Clin Oncol 130: 187-196.
9. Gupta GP, Massague J (2006) Cancer metastasis: building a framework. Cell 127: 679-695.
10. Liotta LA, Kohn EC (2001) The microenvironment of the tumour-host interface. Nature 411: 375-379.
11. Christofori G (2006) New signals from the invasive front. Nature 441: 444-450.
12. Gao CF, Vande Woude GF (2005) HGF/SF-Met signaling in tumor progression. Cell Res 15: 49-51.
13. Buijs JT, Stayrook KR, Guise TA (2011) TGF-beta in the Bone Microenvironment: Role in Breast Cancer Metastases. Cancer Microenviron 4: 261-281.
14. Gherardi E, Birchmeier W, Birchmeier C, Vande Woude G (2012) Targeting MET in cancer: rationale and progress. Nat Rev Cancer 12: 89-103.
15. Ueki T, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E (1997) Expression of hepatocyte growth factor and its receptor c-met proto-oncogene in hepatocellular carcinoma. Hepatology 25: 862-866.
16. Osada S, Kanematsu M, Imai H, Goshima S (2008) Clinical significance of serum HGF and c-Met expression in tumor tissue for evaluation of properties and treatment of hepatocellular carcinoma. Hepatogastroenterology 55: 544-549.
17. Ke AW, Shi GM, Zhou J, Wu FZ, Ding ZB, et al. (2009) Role of overexpression of CD151 and/or c-Met in predicting prognosis of hepatocellular carcinoma. Hepatology 49: 491-503.
18. Wang ZL, Liang P, Dong BW, Yu XL, Yu de J (2008) Prognostic factors and recurrence of small hepatocellular carcinoma after hepatic resection or microwave ablation: a retrospective study. J Gastrointest Surg 12: 327-337.
19. Kaposi-Novak P (2009) [Comparative genomic classification of human hepatocellular carcinoma]. Magy Onkol 53: 61-67.
20. Ogunwobi OO, Liu C (2011) Hepatocyte growth factor upregulation promotes carcinogenesis and epithelial-mesenchymal transition in hepatocellular carcinoma via Akt and COX-2 pathways. Clin Exp Metastasis 28: 721-731.
21. Jiang Y, Xu W, Lu J, He F, Yang X (2001) Invasiveness of hepatocellular carcinoma cell lines: contribution of hepatocyte growth factor, c-met, and transcription factor Ets-1. Biochem Biophys Res Commun 286: 1123-1130.
22. Ogunwobi OO, Wang T, Zhang L, Liu C (2012) Cyclooxygenase-2 and Akt mediate multiple growth-factor-induced epithelial-mesenchymal

transition in human hepatocellular carcinoma. J Gastroenterol Hepatol 27: 566-578.
23. Goyal L, Muzumdar MD, Zhu AX (2013) Targeting the HGF/c-MET pathway in hepatocellular carcinoma. Clin Cancer Res 19: 2310-2318.
24. You H, Ding W, Dang H, Jiang Y, Rountree CB (2011) c-Met represents a potential therapeutic target for personalized treatment in hepatocellular carcinoma. Hepatology 54: 879-889.
25. Chan SL, Yeo W (2012) Targeted therapy of hepatocellular carcinoma: present and future. J Gastroenterol Hepatol 27: 862-872.
26. Zhu K, Kong X, Zhao D, Liang Z, Luo C (2014) c-MET kinase inhibitors: a patent review (2011 - 2013). Expert Opin Ther Pat 24: 217-230.
27. Diaz D, Ford KA, Hartley DP, Harstad EB, Cain GR, et al. (2013) Pharmacokinetic drivers of toxicity for basic molecules: strategy to lower pKa results in decreased tissue exposure and toxicity for a small molecule Met inhibitor. Toxicol Appl Pharmacol 266: 86-94.
28. Santoro A, Simonelli M, Rodriguez-Lope C, Zucali P, Camacho LH, et al. (2013) A Phase-1b study of tivantinib (ARQ 197) in adult patients with hepatocellular carcinoma and cirrhosis. Br J Cancer 108: 21-24.
29. Chiang KC, Tsui KH, Chung LC, Yeh CN, Chen WT, et al. (2014) Celastrol blocks interleukin-6 gene expression via downregulation of NF-kappaB in prostate carcinoma cells. PLoS One 9: e93151.
30. Li T, Xu WS, Wu GS, Chen XP, Wang YT, et al. (2014) Platycodin D induces apoptosis, and inhibits adhesion, migration and invasion in HepG2 hepatocellular carcinoma cells. Asian Pac J Cancer Prev 15: 1745-1749.
31. Fu WM, Zhang JF, Wang H, Xi ZC, Wang WM, et al. (2012) Heat shock protein mediates the effect of 1,3,5-trihydroxy-13,13-dimethyl-2H-pyran [7,6-b] xanthone on mitochondrial apoptosis in hepatocellular carcinoma. J Proteomics 75: 4833-4843.
32. Xue Q, Ding Y, Shang C, Jiang C, Zhao M (2008) Functional expression of LZ-8, a fungal immunomodulatory protein from Ganoderma lucidium in Pichia pastoris. J Gen Appl Microbiol 54: 393-398.
33. Lin YL, Liang YC, Tseng YS, Huang HY, Chou SY, et al. (2009) An immunomodulatory protein, Ling Zhi-8, induced activation and maturation of human monocyte-derived dendritic cells by the NF-kappaB and MAPK pathways. J Leukoc Biol 86: 877-889.
34. Lin CC, Yu YL, Shih CC, Liu KJ, Ou KL, et al. (2011) A novel adjuvant Ling Zhi-8 enhances the efficacy of DNA cancer vaccine by activating dendritic cells. Cancer Immunol Immunother 60: 1019-1027.
35. Martinez-Montemayor MM, Acevedo RR, Otero-Franqui E, Cubano LA, Dharmawardhane SF (2011) Ganoderma lucidum (Reishi) inhibits cancer cell growth and expression of key molecules in inflammatory breast cancer. Nutr Cancer 63: 1085-1094.
36. Liao CH, Hsiao YM, Hsu CP, Lin MY, Wang JC, et al. (2006) Transcriptionally mediated inhibition of telomerase of fungal immunomodulatory protein from Ganoderma tsugae in A549 human lung adenocarcinoma cell line. Mol Carcinog 45: 220-229.
37. Wu CT, Lin TY, Hsu HY, Sheu F, Ho CM, et al. (2011) Ling Zhi-8 mediates p53-dependent growth arrest of lung cancer cells proliferation via the ribosomal protein S7-MDM2-p53 pathway. Carcinogenesis 32: 1890-1896.
38. Wang PH, Yang SF, Chen GD, Han CP, Chen SC, et al. (2007) Human nonmetastatic clone 23 type 1 gene suppresses migration of cervical cancer cells and enhances the migration inhibition of fungal immunomodulatory protein from Ganoderma tsugae. Reprod Sci 14: 475-485.
39. Liang C, Li H, Zhou H, Zhang S, Liu Z, Zhou Q, Sun F. (2012) Recombinant Lz-8 from Ganoderma lucidum induces endoplasmic reticulum stress-mediated autophagic cell death in SGC-7901 human gastric cancer cells. Oncol Rep 27:1079-1089.
40. Wu CT, Lin TY, Hsu HY, Sheu F, Ho CM, et al. (2011) Ling Zhi-8 mediates p53-dependent growth arrest of lung cancer cells proliferation via the ribosomal protein S7-MDM2-p53 pathway. Carcinogenesis 32: 1890-1896.
41. Liao CH, Hsiao YM, Sheu GT, Chang JT, Wang PH, et al. (2007) Nuclear translocation of telomerase reverse transcriptase and calcium signaling in repression of telomerase activity in human lung cancer cells by fungal immunomodulatory protein from Ganoderma tsugae. Biochem Pharmacol 74: 1541-1554.
42. Hsu HY, Hua KF, Wu WC, Hsu J, Weng ST, et al. (2008) Reishi immuno-modulation protein induces interleukin-2 expression via protein kinase-dependent signaling pathways within human T cells. J Cell Physiol 215: 15-26.
43. Hu CT, Cheng CC, Pan SM, Wu JR, Wu WS (2013) PKC mediates fluctuant ERK-paxillin signaling for hepatocyte growth factor-induced migration of hepatoma cell HepG2. Cell Signal 25: 1457-1467.
44. Wu JR, Hu CT, You RI, Ma PL, Pan SM, Lee MC, Wu WS. (2015) Preclinical Trials for Prevention of Tumor Progression of Hepatocellular Carcinoma by LZ-8 Targeting c-Met Dependent and Independent Pathways. PLoS One. 10(1):e0114495.
45.Hu CT, Wu JR, Wu WS (2013) The role of endosomal signaling triggered by metastatic growth factors in tumor progression. Cellular signalling, 25:1539-1545.45.
46.Joffre C, Barrow R, Menard L, Calleja V, Hart IR, Kermorgant S (2011) A direct role for Met endocytosis in tumorigenesis. Nature cell biology, 13:827-837.
47. Haak-Frendscho M, Kino K, Sone T, Jardieu P (1993) Ling Zhi-8: a novel T cell mitogen induces cytokine production and upregulation of ICAM-1 expression. Cell Immunol. 150:101-113.
48.Bastiaan-Net S, Chanput W, Hertz A, Zwittink RD, Mes JJ, Wichers HJ. (2013) Biochemical and functional characterization of recombinant fungal immunomodulatory proteins (rFIPs). Int Immunopharmacol 15:167-175.