LETTER

Reply to Liu: Amino acid 104 asparagine/glutamic acid of p53 is an adaptively selected site for extreme environments in mammals of the Tibet plateau

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In our paper, we substantiate the fact that codon 104 variation is adaptive in highland Tibet plateau mammals (1). Liu's letter (2) claims that codon 104 is not adaptive, which is only based on searching the sequence prediction in National Center for Biotechnology Information (NCBI) without experimental evidence.

Although the asparagine-104 (104N) also occurs in other lowland rodents, this does not mean that this mutation has occurred in the ancestors of rodents. In fact, among the rodents that Liu (2) mentions, all their p53 sequences, except mouse, rat, and gerbil, are based on prediction in NCBI. Notably, the fact that some other species other than rodents also carry 104N argues against the rodent ancestry of 104N. Codon 104 is believed to be adaptive because of its variation between two very close lineages, *Myospalax baileyi* (104N) and *Myospalax cansus* (104S), living in highland and lowland, respectively (1). *M. baileyi* evolved with the Tibet plateau rise and was geographically isolated (3). If this codon is occurring in the ancestors of rodents, there is no reason that *M. baileyi* and *M. cansus* will carry different residues on codon 104. The fact that 104N is not unique to *M. baileyi* does not affect its adaptive role. Our experiments clearly show that mutating serine-106 (106S) into asparagine-106 (106N) in human p53 (corresponding to 104 in *M. baileyi*) did not change the transactivation of Apaf1 and IGFBP3 (figure S5*B* in ref. 1), which were affected by this mutation in *M. baileyi* p53 (figure 2 and figure S5*A* in ref. 1). These results strongly suggest that the 104N of *M. baileyi* p53 plays important roles in adaptive functional changes in cooperation with the context of the *M. baileyi* p53 sequence.

Liu (2) claims that glutamic acid-104 (104E) of p53 is not unique to *Microtus oeconomus* by comparing it with the closely related prairie vole, *Microtus ochrogaster*, which also only has a predicted sequence of p53 in NCBI. In fact, the 104E of *M. oeconomus* shows clearer signs of adaptation because it resembles four fishes (*Barbus barbus*, *Platichthys flesus*, *Tetraodon miurus*, and *Xiphophorus hellerii*) and the squid *Loligo forbesi*, which are hypoxia tolerant (see references 45–47 in ref. 1). The homoplasy of *M. oeconomus* 104E with five hypoxia tolerant aquatic organisms instead of with other mammals clearly shows the convergent evolution of this codon adapting to hypoxia.

As for functional comparison, the human p53 was used as a control because it has been extensively studied. In addition, the genetically very close lineage species *M. cansus* in the lowland was used as another control for *M. baileyi* in the highland, highlighting the importance of codon 104 variation in the same genus (1).

Liu challenges the contribution of 104N of *M. baileyi* p53 because not all target genes were changed on mutation of this site, which is not solid evidence arguing against the adaptive roles of codon 104 variation. As we already mentioned in our paper, the location of codon 104 does not interact directly with DNA (figure S7 in ref. 1); therefore, it should not induce universal changes in transactivation of target genes (1). In fact, the expression patterns of target genes in response to stresses weigh more than that under normal conditions when it comes to environmental adaptation. Our experiments showed that every target gene was affected by codon 104 mutation under all or some stresses (figures 5 and 6 in ref. 1). These results significantly highlight the important roles of codon 104 variation in target gene expression in response to stresses. Thus, there is no reason that codon 104 of p53 in plateau mammals is not adaptive. By contrast, its adaptive nature has been fully confirmed experimentally.

1 Zhao Y, et al. (2013) Codon 104 variation of *p53* gene provides adaptive apoptotic responses to extreme environments in mammals of the Tibet plateau. *Proc Natl Acad Sci USA* 110(51):20639–20644.
2 Liu Z (2013) Codon 104 of p53 is not an adaptively selected site for extreme environments in mammals of the Tibet plateau. *Proc Natl Acad Sci USA* 111:E2357.
3 Tang LZ, et al. (2010) Allopatric divergence and phylogeographic structure of the plateau zokor (*Eospalax baileyi*), a fossorial rodent endemic to the Qinghai-Tibetan Plateau. *J Biogeogr* 37(4):657–668.

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The authors declare no conflict of interest.

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