Author's response to reviews

Title: Li-Fraumeni syndrome with simultaneous osteosarcoma and liver cancer: Increased expression of a CD44 variant isoform after chemotherapy.

Authors:

Go J Yoshida (go-21@lily.ocn.ne.jp)
Yasushi Fuchimoto (yfuchimoto@z5.keio.jp)
Tomoo Osumi (tomo-irie@sa3.so-net.ne.jp)
Hiroyuki Shimada (hshimada@a5.keio.jp)
Seiichi Hosaka (s.hosaka@scchr.jp)
Hideo Morioka (morioka@z3.keio.jp)
Makio Mukai (mukai@a2.keio.jp)
Yohei Masugi (masugi@z6.keio.jp)
Michiie Sakamot (msakamot@z5.keio.jp)
Tatsuo Kuroda (kuroda-t@z8.keio.jp)

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Author's response to reviews: see over
Dear Editors:

Please find attached a revised version of our manuscript “Li-Fraumeni syndrome with simultaneous osteosarcoma and liver cancer: Increased expression of a CD44 variant isoform after chemotherapy”, which we would like to resubmit for publication in BMC cancer as a Case reports.

We believe that it is greatly improved as a result. We would be most grateful if you would consider the accompanying revised manuscript, along with the attached point-by-point response to the reviewers’ earlier criticisms, describing the changes we have made.

We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in BMC cancer.

I look forward to hearing from you at your earliest convenience.

Sincerely,

Yasushi Fuchimoto

Division of Surgery, Department of Surgical Subspecialities
National Center for Child Health and Development
2-10-1 Okura Setagaya-ku
Tokyo 157-8535, Japan
Tel: +81-3-3416-0181
Fax: +81-3-5494-7909
E-mail: yfuchimoto@z5.keio.jp
Response to the comments of Reviewer #1

1. The authors state the reasons for the selection of CD44, had they thought about other CSCs markers?

Response: To be sure, CD133 has widely been recognized as cancer stem cell (CSC) markers for several kinds of tumors. The case of double cancers composed of osteosarcoma and liver cancer in this Li-Fraumeni patient is not an exception. However, there has been no conclusive report on how CD133 affects the functional maintenance of CSC, such as resistance to conventional anti-cancer therapy and the plasticity between quiescence and proliferative state. Furthermore, there is only paraffin-embedded specimens available for research in this case, which is why it was quite difficult to detect the ratio of ALDH-1(high) cells by flow cytometry.

2. Please tell us more information about the function of this variant isoform in the introduction and justify why you chose this CD44 variant isoform (CD44v8-10).

Response: Alternative splicing makes diversity of CD44 isoforms. For instance, CD44v6 interacts with c-Met, the receptor of hepatocyte growth factor (HGF), thereby increasing the survival and proliferative ability of tumor cells. However, the specific aim of this research is to identify the expression change of CD44v8-10, the CSC marker which makes cancer cells resistant to oxidative stress, which is induced by chemotherapy in vivo. As mentioned in the introduction of this paper, CD44v8-10 enhances the reduced glutathione (GSH) synthesis by stabilizing the cystine transporter, xCT. As far as we know, there has been no case reports that demonstrate the ectopic expression of CD44v in double cancers of osteosarcoma and liver cancer during the course of treatment. That is why we regard this case to deserve for publication. We added the following sentence in revised manuscript in page 6, 1para, “For instance, CD44v6 interacts with c-Met, the receptor of hepatocyte growth factor (HGF), thereby increasing the survival and proliferative ability of tumor cells. That is one of the reasons why the expression of the CD44 splice variant CD44v6 is correlated with the metastasis of colon cancer to the liver and a poor clinical prognosis”.

3. The authors described “It is estimated that 70% of individuals with LFS and 8-22% of individuals with LFL syndrome have detectable p53 mutations.” They should be noted that the reference.
Response: We inserted reference [5] in page 5, line 13, as you have pointed out. 

4. Page 5, para 2: "comprehensive" should be "Comprehensive ".

Response: We changed "comprehensive" to "Comprehensive " in page 5, para 2, as you 
have pointed out.

Response to the comments of Reviewer #2

1. This Li-Fraumeni syndrome patient has been confirmed the mutation of P53, so the CD44 
v8-10 expression after chemotherapy is able to understand. However, you should confirm 
the expression of CD44v8-10 in other patients with liver tumors or osteosarcomas receiving 
chemotherapy as negative controls.

Response: We have performed immunohistochemistry (IHC) of the two TP53-intact 
hepatoblastoma specimens using the specific antibody against CD44v8-10 (CD44v). One 
case was the patient of hepatoblastoma who received liver transplantation after 
hepatectomy of the recurrence and received chemotherapy. The other case was the 
hepatoblastoma that was resectable by high dose chemotherapy. We have found that 
CD44v8-10 was not at all expressed in both cases of hepatoblastoma in the absence of p53 
gene mutation after chemotherapy.

2. Figure 3 shows that the expression of CD44v8-10 was detected in the liver tumor and 
osteoarcoma after chemotherapy. CD44v8-10 is generated by ESRP1. Can you confirm 
the expression of ESRP1?

Response: ESRP1 is a major up-stream regulator for the expression of CD44v8-10, so that 
we had already performed IHC using the antibody against human ESRP1 (Sigma). 
Unfortunately, this antibody was not suitable for IHC due to reactions to the non-specific 
antigens. We could not obtain data that shows ESRP1 exclusively in the nucleus. We added 
the following sentence "We have already performed IHC using the specific antibody against 
human ESRP1 and ESRP2. Unfortunately, there was no antibody which was suitable for 
IHC due to reactions to the non-specific antigens, so that we could not obtain data that 
shows ESRP1 or ESRP2 exclusively in the nucleus (data not shown)." in page 11, 1 para.
3. The author described that this patient has been no sign of relapse or metastasis. How much time did it elapsed after last surgery.

Response: We inserted the sentence "To date, there has been no sign of relapse or latent metastasis for about two years after last surgery." in page 9, 1st line.