Role of SFRP1 in NPC Metastasis - Response

Authors and affiliations:

Xian-Yue Ren, Na Liu, Jun Ma

Sun Yat-sen University Cancer Center; State Key Laboratory of Oncology in South China; Collaborative Innovation Center of Cancer Medicine, Guangzhou, PR China

Disclosure of Potential Conflicts of Interest:

No potential conflicts of interest were disclosed.

We would like to thank Shahid Sales and colleagues for their thoughtful letter about our article (1). They raised three important methodologic issues: (i) the isolation of a relatively pure population of tumor cells; (ii) the choice of the housekeeping gene (HKG) for accurate normalization; and (iii) the re-evaluation and validation of the immunohistochemistry score. We appreciate their interest in our study about the role of SFRP1 in nasopharyngeal carcinoma (NPC) metastasis and agree that these important considerations must be addressed.

NPC tissue is characterized by cellular heterogeneity. Therefore, the isolation of a relatively pure population of tumor cells may allow the identification of tumor-specific molecular alterations. Laser-capture microdissection is a useful technique that allows efficient and precise isolation of pure cell populations or even of single cells from complex heterogeneous tissue structures (2). However, tissues from NPC biopsies are usually low in volume and comprise numerous cancer nests separated by stroma. These features greatly hinder laser-capture microdissection. Recent studies, based upon hematoxylin and eosin staining, have reported an alternative method to decrease the bias caused by inclusion of normal tissue—samples are selected in which tumor
tissue comprises the major area for analysis (3). In our study, we performed immunohistochemistry and determined the SFRP1 protein expression by evaluating the staining intensity and the proportion of positive cells in tumor tissue alone.

Accurate normalization is a crucial step for gene expression studies. To this end, the publication in 2009 of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) established guidelines, which recommend that at least two validated reference genes be used for accurate normalization. Subsequently, it became increasingly apparent that a prior test of reference gene stability is also necessary before the genes are chosen. In the past few years, there appears to be a trend toward testing against more reference genes. However, GAPDH is still commonly chosen as a reference gene and has been since it became a standard in the 1980s and early 1990s during the era of Northern blotting. Chapman and colleagues reported that over 72% of studies still use GAPDH, ACTB, or 18S rRNA as single normalizing genes (4). GAPDH, which is a housekeeping gene, has also been widely used to normalize the expression of genes in studies of NPC. With developments in methodology, the geometric means of multiple internal-control genes and the determination of gene stability appear to be setting trends in gene expression studies.

Today, distant metastasis remains the main reason for treatment failure in NPC. Molecular prognostic markers are urgently needed that can predict distant metastasis and guide individualized treatment for patients with NPC. Recently, we reported that a 6-hypermethylated gene panel including SFRP1 was associated with poor survival in patients with NPC (5). It has been reported that SFRP1, which is hypermethylated, can help predict prognosis in various cancers. These findings indicate that SFRP1 should probably be a therapeutic target for cancer.
therapy. However, whether SFRP1 plays a direct role in NPC remains unclear. Immunohistochemistry is an imprecise and empirical method with which to examine the expression of SFRP1 in NPC patients. This is because the immunohistochemistry result depends on the antibody used and pathologists’ expertise. We chose an antibody that had been widely used in other immunohistochemistry staining studies, and all tumor samples were evaluated and re-evaluated by at least two independent pathologists.

Although there are some imperfections, the experimental techniques and the methods we used to analyze our data have been widely used and verified by our peers. Nevertheless, with the development of new methodologies, the design of experiments will require modification in the future.

References:


methylation gene panel as a prognostic biomarker in nasopharyngeal carcinoma. Mol Cancer Ther 2015;14:2864–73.
Role of SFRP1 in NPC Metastasis - Response

Jun Ma, Xian Yue Ren and Liu Na

Cancer Prev Res Published OnlineFirst February 15, 2016.

Updated version
Access the most recent version of this article at:
doi:10.1158/1940-6207.CAPR-15-0398

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerpreventionresearch.aacrjournals.org/content/early/2016/02/13/1940-6207.CAPR-15-0398.citation.
Click on “Request Permissions” which will take you to the Copyright Clearance Center’s (CCC) Rightslink site.