Modern Pathology (2010) 23, 905–907

c-kit gene mutations in adenoid cystic carcinoma are rare

Modern Pathology (2010) **23,** 905–906; doi:10.1038/modpathol.2010.61

To the Editor: It was with great surprise that we read the article entitled 'Identification of *c-kit* gene mutations in primary adenoid cystic carcinoma of the salivary gland' by Vila *et al*,[1] who purported to show a high rate (7/8, 88%) of activating *c-kit* gene mutations mainly in exon 11. In the study published in 1999 that first identified KIT as an up-regulated gene product in adenoid cystic carcinoma (ACC), exons 11 and 17 of *c-kit* were sequenced in 28 cases from the University of Virginia and no activating mutations were found.[2] This study used sequencing of total PCR products to help obviate the detection of PCR artifacts, and the samples were macrodissected and histologically verified to ensure that tumor cells comprised the majority of the sample. As Vila *et al*. also correctly noted, there are three other groups who have also published the results of negative screens for *c-kit* mutations in ACC.[3–6] In addition, we have recent unpublished data from 25 cases of ACC (frozen tissue) obtained from the University of Texas MD Anderson Cancer Center that were subjected to a complete exon sequencing of *c-kit*, in a study performed at the Wellcome Trust Sanger Institute, in which no mutations were found.

The technology utilized by Vila *et al*. included 38 PCR amplification cycles of DNA obtained from formalin-fixed, paraffin-embedded (FFPE) tissues, with the subsequent selection of a few individual colonies (2–5) of cloned PCR products for sequencing. The authors did not report the percentage of clones in which sequence changes were detected. We contend that this approach is inherently susceptible to artifact caused by DNA damage that occurs during FFPE processing of tissue, leading to base substitutions during PCR template replication, especially when limited material is used. This problem of mutation detection in FFPE tissue has been clearly documented.[7] The authors' findings would therefore have been more cogent if standard controls for such analyses had been used and reported, including the sequencing of products of independent PCR amplifications for confirmation and sequencing matched non-neoplastic tissues.[7] Another concern regarding the results in this paper is the fact that several of the tumors had multiple mutations identified in the *c-kit* gene, including silent mutations. More than one activating mutation in a single sample is very rare in *c-kit* in the published literature, with only a handful of instances in over 2000 mutations reported, with the majority of these occurring during the development of resistance to imatinib (COSMIC).[8] The high incidence of multiple point mutations reported by Vila *et al* thus further points to the possibility of technical artifact. The authors described their technique as 'more sensitive' than those used in other studies, but, in contrast, we believe the previous studies were designed and controlled to prevent artifact and were perfectly adequate to detect mutations that were present in the majority of tumor cells in tissue samples.

The authors characterized the data in the literature regarding the activity of KIT antagonists in treating patients with ACC as 'conflicting'. We, however, argue that, except for two anecdotal studies that they cite,[9] the published data from prospective clinical studies examining the role of anti-KIT targeted therapy (imatinib) in ACC show a very low rate of objective response (1 in 42 subjects, see the table). These clinical data, together with the preponderance of sequencing data, suggest that KIT as a driver mutation in ACC is, at best, rare.

Subjects enrolled	*Response reported*	*Reference*
11	0	Heinrich *et al*[10]
6	1	Faivre *et al*[11]
15	0	Hotte *et al*[12]
10	0	Pfeffer *et al*[13]

In conclusion, studies from FFPE samples are prone to artifacts and, as such, should be validated in fresh frozen material where possible. In this instance, there is a marked lack of corroborative support for a high frequency of activating KIT mutations in ACC in the data generated by several independent studies in such samples. We would therefore suggest that, on balance, there is compelling evidence to support only a very minor role, if any, for activating KIT mutations in ACC, and that caution should be exercised while drawing conclusions for therapeutic application of the data presented by Vila *et al*.

Christopher A Moskaluk[1], Henry F Frierson Jr[2], Adel K El-Naggar[3] and P Andrew Futreal[4]
[1]Department of Pathology, Biochemistry & Molecular Genetics, University of Virginia, Charlottesville, VA, USA; [2]Department of Pathology, University of Virginia, Charlottesville, VA, USA; [3]Department of Pathology and Head and Neck Surgery, University of Texas MD Anderson Cancer Center, Houston, TX, USA and [4]Cancer Genome Project, Wellcome Trust Sanger Institute, London, UK
E-mail: cam5p@virginia.edu

References

1 Vila L, Liu H, Al-Quran SZ, *et al.* Identification of c-kit gene mutations in primary adenoid cystic carcinoma of the salivary gland. Mod Pathol 2009;22:1296–1302.
2 Holst VA, Marshall CE, Moskaluk CA, *et al.* KIT protein expression and analysis of c-kit gene mutation in adenoid cystic carcinoma. Mod Pathol 1999;12: 956–960.
3 Jeng YM, Lin CY, Hsu HC. Expression of the c-kit protein is associated with certain subtypes of salivary gland carcinoma. Cancer Lett 2000;154:107–111.
4 Sorensen KB, Godballe C, de Stricker K, *et al.* Parotid carcinoma: expression of kit protein and epidermal growth factor receptor. J Oral Pathol Med 2006;35: 286–291.
5 Lin CH, Yen RF, Jeng YM, *et al.* Unexpected rapid progression of metastatic adenoid cystic carcinoma during treatment with imatinib mesylate. Head Neck 2005;27:1022–1027.
6 Aubry MC, Heinrich MC, Molina J, *et al.* Primary adenoid cystic carcinoma of the lung: absence of KIT mutations. Cancer 2007;110:2507–2510.
7 Marchetti A, Felicioni L, Buttitta F, *et al.* Assessing EGFR mutations. N Engl J Med 2006;354:526–528.
8 Catalogue of Somatic Mutations in Cancer [database on the Internet] 2009. Wellcome Trust Sanger Institute [cited 2009]. Available from http://www.sanger.ac.uk/genetics/CGP/cosmic/.
9 Alcedo JC, Fabrega JM, Arosemena JR, *et al.* Imatinib mesylate as treatment for adenoid cystic carcinoma of the salivary glands: report of two successfully treated cases. Head Neck 2004;26:829–831.
10 Heinrich MC, Joensuu H, Demetri GD, *et al.* Phase II, open-label study evaluating the activity of imatinib in treating life-threatening malignancies known to be associated with imatinib-sensitive tyrosine kinases. Clin Cancer Res 2008;14:2717–2725.
11 Faivre S, Raymond E, Casiraghi O, *et al.* Imatinib mesylate can induce objective response in progressing, highly expressing KIT adenoid cystic carcinoma of the salivary glands. J Clin Oncol 2005;23:6271–6273.
12 Hotte SJ, Winquist EW, Lamont E, *et al.* Imatinib mesylate in patients with adenoid cystic cancers of the salivary glands expressing c-kit: a Princess Margaret Hospital Phase II Consortium Study. J Clin Oncol 2005;23:585–590.
13 Pfeffer MR, Talmi Y, Catane R, *et al.* A phase II study of imatinib for advanced adenoid cystic carcinoma of head and neck salivary glands. Oral Oncol 2007;43:33–36.

Response to Moskaluk *et al*

Modern Pathology (2010) **23,** 906–907; doi:10.1038/modpathol.2010.64

To the Editor: It is with great interest that we read the comments by Moskaluk *et al*[1] in relation to our paper titled 'Identification of *c-kit* gene mutations in primary adenoid cystic carcinoma of the salivary gland' published in the October 2009 issue of *Modern Pathology*. We appreciate the comments from the authors, who are well-established investigators in the field. We understand the authors' concerns regarding the introduction of artifactual mutations using formalin-fixed paraffin-embedded tissue (FFPE);[2] however, as the study by Marchetti *et al* illustrates, these artifacts are likely to be observed when *very small amounts* of starting DNA (5 ng) are isolated and subjected to *multiple* PCR amplifications. As Marchetti *et al* point out, 'the occurrence of these artifacts can be prevented with the use of larger amounts of template DNA (at least 1 μg of DNA recovered from paraffin).' In our study, we measured the DNA content obtained after extraction to ensure that all tumor samples contained greater than 1.5 μg of starting template DNA. Moreover, tissue samples (which were obtained from large surgical resections and not small biopsies) were microdissected to minimize contamination from surrounding normal tissue and were subjected to a *single* PCR reaction, using a methodology similar to that outlined by Antonescu *et al*.[3]

Although the finding of more than one exon mutation in the *c-kit* gene is rare as Moskaluk *et al* note, ours is not the first study to report multiple *c-kit* mutations within the same tumor and loss or addition of mutations in primary versus metastatic tumor.[4,5] Multiple mutations were quite common in the series of Andersson *et al*, who utilized subcloning of PCR products for exons 12–15 and direct sequencing of exons 9–11 from fresh-frozen tissue of gastrointestinal stromal tumors (GISTs).[4] In this study, six of nine tumors with mutations in exons 11 had one or two additional mutations in exons 14 and 15. In addition, different mutations were found in the primary tumor versus secondary tumor, indicating the complexity between *c-kit* mutations and clinical behavior. These findings suggest the heterogeneity of tumor cells found within the same population and existence of subpopulations of tumor cells with different mutations. These subpopulations would not be detected by direct sequencing of PCR products, which requires the mutation subpopulation to consist of at least 15–20% of the total PCR product.

Although we cannot exclude with absolute certainty the introduction of artifactual mutations in this type of analysis, we believe these represent rare events. In fact, in our study, the majority of clones