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Subclassification of squamous cell carcinomas of the head and neck based on HER/ErbB and c-MET receptor protein expression and activation profiles.

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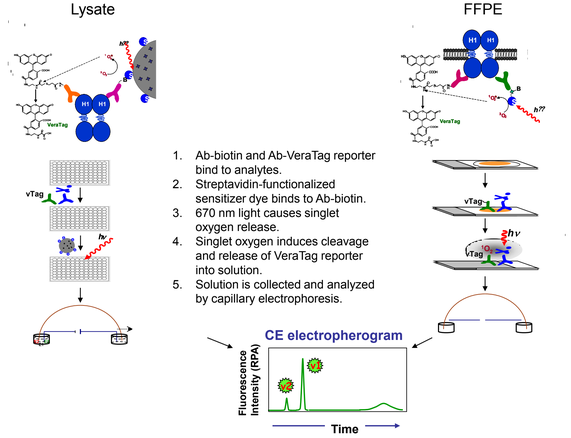
Abstract

The response rates of HER1 antibody monotherapy (~13%) is comparable to the added response rate of HER1 antibody therapy when used in combination with chemotherapy (~16%) in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck (SCCHN). Measures of HER1/ErbB1/EGFR protein levels by immunohistochemistry (IHC) or gene copy number by fluorescent in-situ hybridization (FISH) do not correlate with response, therefore, alternative gene or protein expression signatures are needed to identify patients most likely to respond to HER1 targeted therapies. Previously, quantitative VeraTag proximity immunoassays performed on formalin-fixed, paraffin imbedded SCCHN tumor tissue identified an activated HER1 signature (HER1-HER1 homodimers; HER1pY1173) that was significantly more pronounced in SCCHN compared to other carcinoma of the head and neck. In this study, we analyzed the expression of HER1, 2, 3 and c-MET along with the activation of HER1 and HER3 in 56 SCCHN tumors. Our results identified two distinct protein expression profiles: an activated HER1 profile, and a second profile characterized by the different expression levels of HER2, HER3, phosphorylated HER3, and c-MET relative to activated HER1. Our working hypothesis is that the first profile correlates with sensitivity to HER1 targeted therapies while the second profile correlates with either sensitivity or resistance to HER1 targeted therapies. HER1 protein levels measured by VeraTag assay varied over a ~20-fold dynamic range, and significantly correlated with HER1 IHC staining and mRNA levels determined by qRT-PCR. HER2 and HER3 protein expression spanned a 15- to 20-fold range, and correlated with mRNA levels. Several of the highest HER1 expressing tumors have increased gene copy number; however, similar high values were measured in tumors without amplification, indicating that additional mechanisms of high HER1 expression exist in SCCHN. Although high HER1 activation measured by HER1-HER1 dimer and HER1pY1173 correlates with HER1 levels (r=~0.6, p<0.05), and are associated with the highest levels of HER1 expression measured by multiple methods, not all high HER1 expressing tumors displayed highly activated HER1. These observations may explain why total HER1 expression in SCCHN tumors, determined by IHC or FISH, does not correlate with response to targeted therapies. On the other hand, approximately 16% of the SCCHN tumors studied here (9/56) exhibit a highly activated HER1 profile; similar in magnitude to the response rate seen with HER1 targeted antibody therapy. Future studies are designed to further validate the HER1 activation signature as a predictor of response to HER1 targeted therapies and/or the attenuation of response due to HER2, HER3 or c-MET expression.

VeraTag Technology



1. Ab-biotin and Ab-VeraTag reporter bind to analytes.
2. Streptavidin-functionalized sensitizer dye binds to Ab-biotin.
3. 670 nm light causes singlet oxygen release.
4. Singlet oxygen induces cleavage and release of VeraTag reporter into solution.
5. Solution is collected and analyzed by capillary electrophoresis.

1. Head and Neck Tumors

The squamous cell carcinoma of the head and neck tumors used for this study were separated into two groups, 5-38, and 51-95, which were analyzed by two separate sets of assay testing and were batch normalized to allow direct comparison of all values.

Sample No.	Tissue
5	Lip
7*	Gum
10	Larynx
11	Larynx
12	Larynx
13	Larynx
14**	Larynx
15**	Larynx
16	Larynx
17	Larynx
19	Larynx
23	Oropharynx
29	Maxillary sinus
30	Mouth
31	Larynx
35	Larynx
36	Larynx
37	Tongue
38	Larynx

N = 19
18 unique

Sample No.	Tissue
51	Larynx
52	Larynx
53	Larynx
55	Larynx
56	Larynx
57	Larynx
58	Tongue
59	Tongue
60	Larynx
61	Lip
62**	Gum
64	Larynx
66	Larynx
68	Tongue
69	Larynx
72	Larynx
73	Lip
74	Oral Mucosa
75	Mouth
76	Tongue
77	Oral Mucosa
78	Larynx

Sample No.	Tissue
81	Tongue
82	Pharynx
83	Pharynx
84	Tongue
85	Larynx
86	Lip, Nos
87	Oral Muscosa
88	Tongue
89	Tongue
90	Tongue
91**	Gingiva
92	Gingiva
93	Lip, Nos
94	Lymph Node, tongue
95	Lymph Node, tonsil

N = 37
36 unique

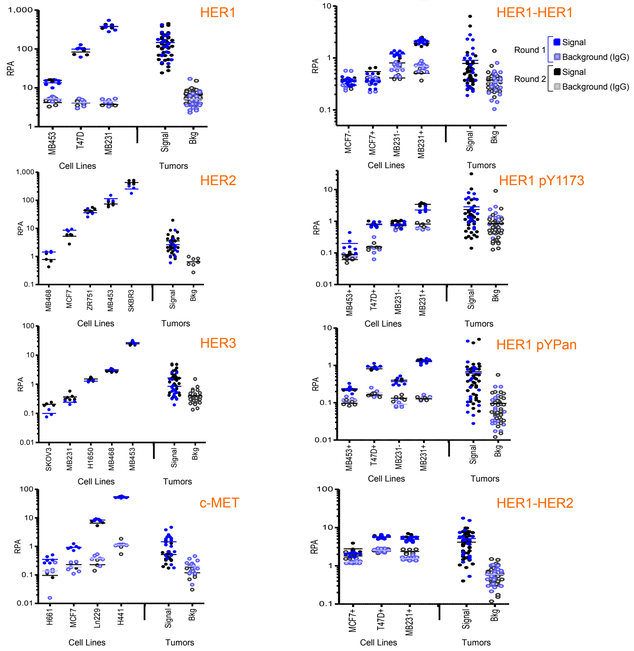
Tissue Distribution
26 Larynx (48%)
10 Tongue (19%)
6 Mouth (11%)
5 Lip (9%)
4 Gum (7%)
2 Pharynx (4%)
1 Oropharynx (2%)
2 Lymph nodes

N=56 total SCCHN tumors

* Highly activated HER1 tumors
** Tumor sample from the same patient

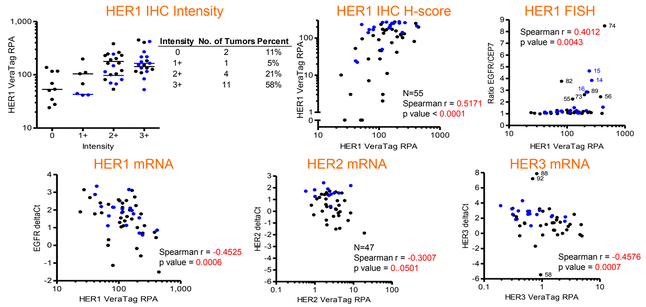
2. VeraTag Assays and Performance

Control cell lines were assayed together with the tumors. The distribution of the assay signal (black, blue) is shown together with the isotype control background (ITC, gray) for both the tumors and control cell lines. Tumor samples were macro-dissected to ≥70% tumor and ≤10% non-tumor elements.



3.Correlations with IHC, FISH, and mRNA

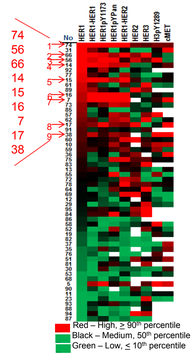
VeraTag assays for the head and neck squamous cell carcinomas were compared to mRNA, FISH, and IHC from the same tumor. Significant correlations ($p < 0.05$) were found for all HER1-based comparisons. HER2 and HER3 VeraTag correlates with mRNA with p= 0.05 and 0.016, respectively.



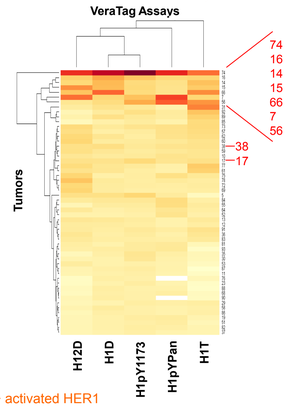
4. HER1 Activation in SCCHN

Three different analyses of the protein expression profiles for the SCCHN tumors obtained from the VeraTag assays define an activated HER1 signature for 7-9/56 (13-16%) tumors.

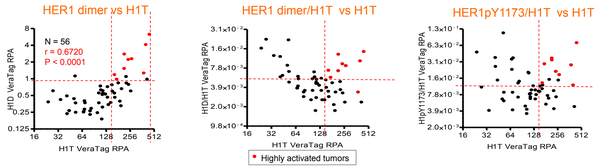
A. Heat Map



B. Hierarchical Cluster Analysis



C. 2 variables required to stratify tumors: H1T + activated HER1



5. VeraTag Correlations in SCCHN

All pairwise comparisons between the VeraTag assays of the combined 56 SCCHN tumors. The Spearman correlation coefficients having significant p values ($p < 0.05$) are shown in red. All pairwise comparisons within HER1 and the HER1 activated assays are significant. A correlation analysis was also performed on those tumors having assay S/B > 50%, giving similar results.

Combined

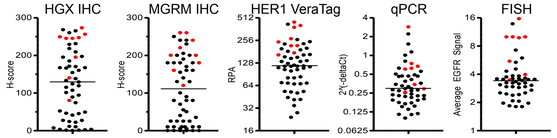
	H1D	H1pY1173	H1pPan	H12D	HER2	HER3	cMET
HER1	0.6694	0.6181	0.4668	0.6034	0.1549	0.0628	0.1428
H1D		0.6291	0.4949	0.6214	0.3353	0.0749	0.2326
H1pY1173			0.5873	0.5024	0.1970	-0.0727	0.3531
H1pPan				0.5780	0.4081	0.2336	0.0280
H12D					0.3002	0.1363	0.1474
HER2						0.3132	-0.0949
HER3							-0.3492

Combined with bkg <50%

	H1D	H1pY1173	H1pPan	H12D	HER2	HER3	cMET
HER1	0.7234	0.5344	0.3568	0.6034	0.1186	-0.0294	0.0293
H1D		0.7169	0.5788	0.6214	0.0243	0.0823	0.1835
H1pY1173			0.4429	0.3692	0.2231	-0.2673	0.3977
H1pPan				0.5443	0.3162	0.2326	-0.1207
H12D					0.2827	0.0757	-0.0476
HER2						0.2721	-0.1645
HER3							-0.4515

6. Activated HER1 distribution vs HER1

The activated HER1 tumors have a large distribution in all HER1 measurements, from the highest expression down to the median (black line). Consequently, the majority of tumors in this range (~19 of 28) are not highly activated. A cut point for any HER1 measurement would not stratify those patients having tumors with high levels of activated HER1 whereas a combination of HER1 total expression and activated HER1 can stratify tumors.*



•Note: Combining HER1 expression measurements also does not stratify the highly activated HER1 tumors. This includes combinations of FISH gene copy numbers with VeraTag, IHC, and qPCR mRNA, or any other combinations of those measurements.

Summary

- Quantitative FFPE assays were developed using the VeraTag technology to measure protein levels of the HER family and c-Met receptor tyrosine kinases in 56 head and neck squamous cell carcinomas.
- Using macro-dissected samples, a range of HER1 protein levels were measured by VeraTag assays in >90% of the SCCHN tumors, whereas a smaller subset expressed the highest levels of HER1 and activated HER1 (HER1-HER1 dimer and HER1pY1173). More than half the tumors express HER2, HER3, and c-MET.
- The HER1 VeraTag total protein measurements significantly correlated with HER1 IHC and mRNA levels determined by qPCR. FISH HER1 gene copy ratio to CEP7 correlated with HER1 VeraTag levels where there is HER1 amplification, however, high HER1 protein expression is observed in the absence of gene amplification indicating alternative regulatory mechanisms.
- Three methods of analysis identify an activated HER1 signature in a small subset of tumors (7-9/56 = ~13-16%), a percentage consistent with the patient response rate of HER1-targeted therapy (cetuximab) in SCCHN (~13-16%- Vermorken et al. N Engl J. Med. 2008, 359, 1116; Vermorken et al. J. Clin. Oncol. 2007, 25: 2171)
- Tumors with the activated HER1 signature do not stratify as a single group with standard measurements of HER1, consistent with the lack of correlation of HER1 IHC and FISH with response to targeted HER1 therapies. The association of these signatures with prognosis and drug responses will be evaluated in future studies with clinical samples.