

# Comparative analysis of the DT40 and MCF-10A/HepG2 cisplatin signatures in hepatocellular carcinomas and esophageal adenocarcinomas

The experimental single-nucleotide-substitution (SNS) mutational signature of cisplatin in MCF-10A and HepG2 cells is very different from SNS signatures previously published for *Caenorhabditis elegans* (Meier et al. 2014) and the chicken B-cell line DT40 (Szikriszt et al. 2016). However, the dinucleotide substitution (DNS) pattern in cisplatin-treated DT40 cells did in fact resemble the MCF-10A/HepG2 DNS signature (Supplemental Fig. S28B). Therefore, we examined the human hepatocellular carcinoma (HCC) and esophageal adenocarcinoma (ESAD) data for presence of the DT40 SNS cisplatin signature and assessed whether, compared to the MCF-10A/HepG2 signature, it was effective for identifying cisplatin exposure.

## Approach

We used the mSigAct signature.presence.test to assess presence of the DT40 SNS cisplatin signature in the HCCs and ESADs we had previously analyzed for presence of the MCF-10A/HepG2 signature.

We carried out the following tests (in which DT40 denotes the DT40 SNS signature).

Test 1: DT40 against COSMIC Signatures 1, 4, 5, 6, 12, 16, 17, 22, 23, and 24.

Test 2: DT40 against the above COSMIC signatures plus the MCF-10A/HepG2 signature

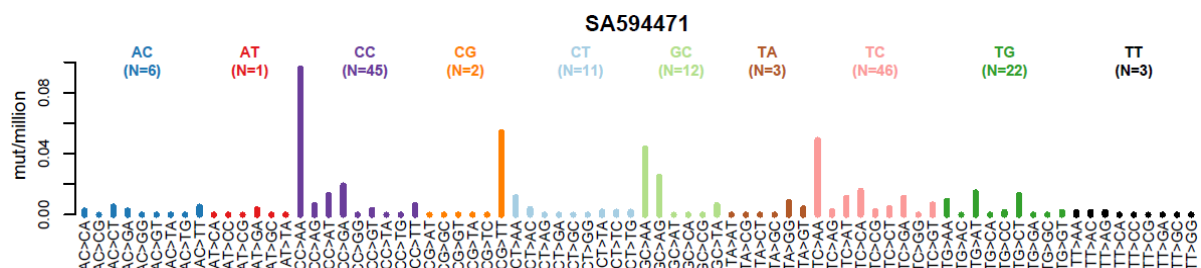
Test 3: The MCF-10A/HepG2 signature against the above COSMIC signatures plus DT40

## Results

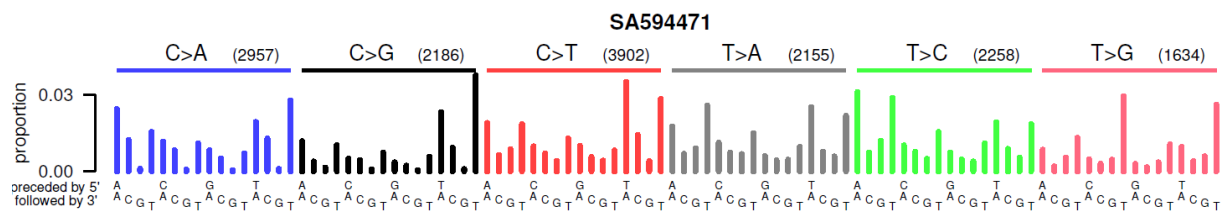
Results are summarized in the table at the end of this document.

Here we discuss the conclusions from these analyses:

**Test 1** assesses how often inclusion of the DT40 signature (absent the MCF-10A/HepG2 signature) provides a statistically better reconstruction than one without it. Of the 4 tumors showing evidence for the DT40 signature in this test, 3 had been identified with the MCF-10A/HepG2 signature (main text Table 1). The new tumor was SA594471, a post-chemotherapy sample from the ESAD cohort. Inspection of the DNS spectrum of this sample (figure below) provided little evidence of the cisplatin-associated DNS pattern observed in DT40 and MCF-10A/HepG2. Consistent with this, DNS ssNMF attributed only 22.5% of the DNSs SA594471 to the MCF-10A/HepG2 cisplatin DNS signature.



Visual inspection of the SNS spectra of samples found significant for the DT40 signature (RK028, RK056, SA594320 and SA594471) did not show the CC>CA peaks that are a striking feature of the DT40 SNS signature (Supplemental Fig. S28A plus figure below).



**Test 2** assesses whether adding the DT40 signature to a set of signatures already containing the MCF-10A/HepG2 signature provides a significantly better reconstruction. The table shows that this is never the case.

**Test 3**, conversely, assesses whether adding the MCF-10A/HepRG signature to a set of signatures containing the DT40 signature provides a significantly better reconstruction. The Table shows that for 9 of the 13 tumours previously identified as cisplatin positive, this is indeed the case.

## Conclusion:

The original mSigAct signature presence tests of the MCF-10A/HepG2 cisplatin signature (main text Table 1 and the table in this file) combined with the results of Tests 1, 2, and 3 above indicate that the MCF-10A/HepG2 signature is more effective at identifying cisplatin-mutagenized tumors. Additional evidence that the MCF-10A/HepG2 signature recapitulates the cisplatin signature in human tumors is the presence of some tumors that have spectra in which the MCF-10A/HepG2 signature is discernible by inspection (for example HK034, main text Fig. 4A), which we believe reflects strong mutagenesis by cisplatin. Conversely, we are unaware of any human tumor or previously extracted mutational signature (Wellcome Trust Sanger Institute 2016) with the  $\underline{CC}>\underline{CA}$  peaks that are prominent in the DT40 cisplatin signature.

## References

- Meier B, Cooke SL, Weiss J, Bailly AP, Alexandrov LB, Marshall J, Raine K, Maddison M, Anderson E, Stratton MR et al. 2014. *C. elegans* whole-genome sequencing reveals mutational signatures related to carcinogens and DNA repair deficiency. *Genome Res* **24**: 1624-1636.
- Szikriszt B, Poti A, Pipek O, Krzystanek M, Kanu N, Molnar J, Ribli D, Szeltner Z, Tusnady GE, Csabai I et al. 2016. A comprehensive survey of the mutagenic impact of common cancer cytotoxics. *Genome Biol* **17**: 99.
- Wellcome Trust Sanger Institute. 2016. COSMIC, Catalog of Somatic Mutations in Cancer - Signatures of Mutational Processes in Human Cancer, <http://cancer.sanger.ac.uk/cosmic/signatures>.

**Table. mSigAct analysis of the DT40 cisplatin signature in HCCs and ESADs compared to the MCF-10A/HepG2 cisplatin signature**

Sample	Cisplatin treated	total SNSs	MCF-10A/HepG2 cisplatin signature*	DT40 (Test 1)	DT40 (with cisplatin) (Test 2)	Cisplatin (with DT40) (Test 3)
			pval**	pval	pval	pval
RK056	Yes	17085	4.4E-26	2.5E-09	2.7E-01	1.5E-18
RK028	Yes	20792	1.5E-21	3.9E-08	2.2E-01	3.1E-15
RK074	Yes	22406	2.5E-18	4.7E-03	1.0E+00	1.4E-16
RK241	Yes	10610	5.1E-16	1.3E-05	3.0E-01	4.6E-12
RK256	Yes	11240	2.4E-09	5.6E-04	2.6E-01	5.8E-07
RK205	Yes	10406	3.0E-06	1.1E-01	9.6E-01	1.1E-05
RK140	Yes	10132	2.5E-04	2.8E-02	3.0E-01	1.9E-03
RK072	Yes	8893	4.9E-04	1.6E-02	1.9E-01	4.4E-03
HK034	Unknown	7844	1.3E-11	8.9E-03	9.7E-01	1.3E-10
RK309	--	4785	2.3E-05	1.0E+00	1.0E+00	2.3E-05
RK047	--	8345	1.1E-04	1.5E-01	1.0E+00	3.3E-04
RK223	--	10680	1.4E-04	1.3E-02	3.3E-01	2.3E-03
SA594320	Yes	23483	9.6E-19	7.6E-11	8.8E-02	4.9E-10
SA594775	Yes	14967	4.3E-06	6.0E-04	1.7E-01	8.0E-04
SA594557	Yes	7433	1.0E-05	1.2E-04	8.4E-02	5.6E-03
SA594471	Yes	15092	5.6E-04	8.5E-06	1.7E-03	1.6E-01

\* Analysis as in main text Table 1.

\*\* Bonferroni level of significance is 1.5E-04 (0.05/342) for the HCCs and 3.6E-04 (0.05/140) for the ESADs , significant pvals are shown in green