High incidence of protein-truncating *TP53* mutations in BRCA1-related breast cancer

Henne Holstege, Simon A. Joosse, Conny Th. M. van Oostrom, Petra Nederlof, Annemieke de Vries, and Jos Jonkers

Supplementary Results

Previous comparisons of p53 mutations from BRCA1/2 related breast cancers with those found in sporadic tumors reported in the IARC-TP53 database showed that p53 mutations found BRCA1/2 related breast tumors were rare non-hotspot p53 mutations, and silent, tandem and multiple p53 mutations were common in BRCA1/2 related breast tumors (1-4). In contrast to p53 hotspot mutations, these BRCA-specific p53 mutations did not localize to the common DNA-binding domain, but instead localized to ‘the opposite side of the DNA binding surface’. Compared to the sporadic tumors reported in the IARC database, BRCA1/2 tumors were found to a have a greater amount of p53 mutations at A:T base pairs and more tandem CC>TT mutations. To see if we could find similar properties in p53 mutations found in our BRCA1-related breast tumors, we performed an analysis of the p53 mutations found in our tumor groups.

To assess the rarity of p53 mutations in our tumor groups, we calculated the fraction of p53 mutations that have not been found previously in breast cancer

according to the TP53 IARC database[1] (Supplementary Table 2). Mutations new to breast cancer concerned 52.9% (27/51) and 52.2% (35/67) of the p53 mutations in BRCA1-associated and sporadic tumors respectively (Supplementary Table 4A). P53 mutations new to cancer in general involved 11.8% (6/51) of all p53 mutations in BRCA1 tumors and 11.9% (8/67) of p53 mutations in sporadic tumors. These new p53 mutations are either neutral missense mutations or silent mutations that map primarily outside the DNA binding domain (Supplementary Table 2). However, for reasons of completeness we included them in our analysis. Overall, our data show no significant difference in the occurrence of novel p53 mutations in BRCA-associated tumors compared with sporadic tumors (P=1 for both comparisons, Bonferroni corrected Fisher's Exact Test).

Next, to assess the prevalence of breast cancer specific p53 mutations we counted those p53 mutations for which the tissue of origin was breast cancer in more than 25% of the cases in the IARC database (Supplementary Table 4A). BRCA1 related tumors do not have significantly more breast cancer specific p53 mutations than sporadic breast tumors, as 9.8% (5/51) p53 mutations in BRCA1 tumors were breast cancer specific compared with 6.0% (4/67) p53 mutations in sporadic tumors. Also, the domain functions and residue functions of p53 mutations had a similar distribution in the BRCA1 tumor group and the sporadic tumor group (Supplementary Table 4B). When assessing BRCA1-specific p53 mutations, we identified 1 BRCA1-specific p53 mutation (R213X) and 2 sporadic specific p53 mutations (P151P and G187S);

[1] www-p53.iarc.fr/TumorCriteria.asp, somatic mutations search

however, most mutations that occurred in multiple tumors occurred in both BRCA1 and sporadic breast tumors (Supplementary Table 5).

To determine the amount of hotspot and non-hotspot missense mutations in each group, we used the list of hotspot p53 mutations found by Walker et al. (5). The BRCA1 tumors had approximately the same proportion of hotspot missense p53 mutations as those found in the sporadic tumors: 21.6% (11/51) vs. 25.4% (17/67) respectively (Table 3A, Figure 3). Similarly, the fraction of the most common hotspots was very similar between the two groups: 17.6% (9/51) for the BRCA1 tumors and 20.9% (14/67) for the sporadic tumors. Deleterious missense mutations that were non-hotspot mutations were found in approximately the same proportions in the BRCA1 and sporadic tumor groups: 11.8% (6/51) vs. 14.9% (10/67) (Table 3A).

One of the most profound differences previously reported for BRCA-related p53 missense mutations involved their increased occurrence at A:T base pairs. However, we find that only 2.0% (1/51) of the p53 mutations in our BRCA1 tumors occurred at A:T base pairs, compared to 6.0% (4/67) of the p53 mutations found in sporadic tumors (Supplementary Table 4C). In sum, our analysis of p53 mutations found in our BRCA1 and sporadic tumor groups does not confirm previous reports on specificity of BRCA1- dependent p53 mutations.

Reference List

1. Crook T, Brooks LA, Crossland S, et al. p53 mutation with frequent novel codons but not a mutator phenotype in BRCA1- and BRCA2-associated breast tumours. Oncogene 1998;17:1681-9.

2. Gasco M, Yulug IG, Crook T. TP53 mutations in familial breast cancer: functional aspects. Hum Mutat 2003;21:301-6.

3. Greenblatt MS, Chappuis PO, Bond JP, Hamel N, Foulkes WD. TP53 mutations in breast cancer associated with BRCA1 or BRCA2 germ-line mutations: distinctive spectrum and structural distribution. Cancer Res 2001;61:4092-7.

4. Smith PD, Crossland S, Parker G, et al. Novel p53 mutants selected in BRCA-associated tumours which dissociate transformation suppression from other wild-type p53 functions. Oncogene 1999;18:2451-9.

5. Walker DR, Bond JP, Tarone RE, et al. Evolutionary conservation and somatic mutation hotspot maps of p53: correlation with p53 protein structural and functional features. Oncogene 1999;18:211-8.