S3. *p53* MUTATIONS IN ENDOMETRIAL TUMOURS FROM TAMOXIFEN-TREATED WOMEN

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Tamoxifen treatment causes an increased risk of endometrial cancer in breast cancer patients and healthy women taking the drug as a chemopreventive agent. The mechanisms responsible for tamoxifen-induced carcinogenesis in humans are presently unknown and there is much interest in identifying whether DNA damage might be a contributing factor. In rats, hepatocarcinomas arise as a consequence of high levels of tamoxifen DNA adduct formation in liver tissue. Furthermore, low levels of tamoxifen DNA binding has been detected in endometrial and myometrial tissue of women administered a single dose of ^{14}C-labelled tamoxifen [1]. The major dG-N^2-tamoxifen adducts formed in rat liver have also been reported in endometrial DNA of some patients receiving long-term tamoxifen treatment [2], although this has not been confirmed by others [3]. Valuable insights into mechanisms of tumour development and the significance of DNA adduct formation can be gained through the analysis of sequence alterations in the *p53* gene of tumours, which can reveal characteristic mutational signatures.

Preliminary results reported by Welsh and colleagues [4] demonstrated that endometrial tumours in tamoxifen-treated women contained an excess of G $\rightarrow$ A transitions at non-CpG sites in the *p53* gene, compared with tumours in untreated women. However, these findings have not been substantiated. Furthermore, rather than transition mutations, the predominant mutation induced in transgenic animals by tamoxifen treatment is typically G $\rightarrow$ T transversions. In addition, replication of single dG-N^2-tamoxifen adducts in cultured mammalian cells primarily causes G $\rightarrow$ T transversions, although smaller numbers of G $\rightarrow$ A transitions are also evident [5]. In order to clarify whether tamoxifen induces a characteristic mutation spectrum in human tumours, we have examined sequence changes in exons 4–9 of *p53* in endometrial tumour samples from six women treated with tamoxifen (20 mg/day) and four untreated patients, using polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) analysis.

Current results indicate the presence of sequence alterations in 5/6 samples from tamoxifen-treated patients and only 1/4 from control patients, as evidenced by the detection of shifted bands. Potential mutations were detected in exons 4, 5 and 8. Five samples exhibited shifted bands in exon 4, which were extracted from the SSCP gels and PCR amplified. Sequence analysis of two samples, from both a tamoxifen-treated and control patient, revealed the presence of two single base substitutions, a C $\rightarrow$ A transversion located within intron 3 and a G $\rightarrow$ C transversion at codon 72. This G $\rightarrow$ C substitution is a common known polymorphism causing an arginine to proline amino acid change, and was also observed as the only alteration in a third sample, from a patient that had received tamoxifen. The remaining two tumours with potential mutations in exon 4 have yet to be confirmed by sequencing. Only two samples, both from tamoxifen-treated patients, exhibited mobility shifts in exon 8, each due to the presence of a single base substitution. One contained a G $\rightarrow$ A transition at codon 273, a common *p53* mutational hotspot in many cancers, which would produce an arginine to histidine amino acid change. The other tumour contained a G $\rightarrow$ T transversion at codon 294 that would substitute a stop codon in place of a glutamic acid residue. A shifted banding pattern was observed in exon 5 of just one sample, from a tamoxifen-treated patient and this is currently being analysed to identify the specific sequence changes.

Initial results indicate there may be an increased frequency of *p53* mutations in endometrial tumours from tamoxifen-treated women, but this remains to be confirmed by sequence analysis. Ultimately, the methodology developed in this pilot study will be used for screening the larger numbers of endometrial tumours necessary to determine conclusively whether tamoxifen induces characteristic types and patterns of mutations in the *p53* gene.

References

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