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Bioinformatic processing to identify single nucleotide polymorphism that potentially affect Ape1 function

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ABSTRACT

Inactivation of DNA damage response mechanisms is associated with several disease syndromes, including cancer, aging and neurodegeneration. A major corrective pathway for alkylation or oxidative DNA damage is base excision repair (BER). As part of an effort to identify variation in DNA repair genes, we used the expressed sequence tag (EST) database to identify amino acid variation in Ape1, an essential gene in the BER repair pathway. Nucleotide substitutions were considered valid only if the amino acid changes were observed in at least two independent EST sequencing runs (i.e. two independent EST reports). In total eighty amino acid variants were identified for the Ape1 gene. Using software tools SIFT and PolyPhen, which predict impacts of amino acid substitutions on protein structure and function, twenty-six variants were predicted by both algorithms to be deleterious to protein function. Majority of these intolerant mutations such as V206C and F240S, lie within the core of the protein and may affect the stability and folding of Ape1, or in the case of N212H, N212K, and Y171N, are close to the enzyme's active site and could drastically affect its function. A few of the intolerant mutations, i.e., G178V and E217R, are surface residues and are far from the active site, and as such, the predicted effect on Ape1 stability or function is not evident. These variants are reagents for further protein function studies and molecular epidemiology studies of cancer susceptibility.

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1. Introduction

Whole genome association studies have recently been proposed as a powerful approach in order to detect numerous subtle genetic effects that may underlie susceptibility to genotoxic exposures as well as common diseases [1–3]. Unlike linkage studies, which look for co-inheritance of chromosomal regions with disease families, association studies look at differences in the frequency of genetic variation between unrelated individuals and controls. Such studies have been used to test the involvement of candidate genes in disease and to refine the location of disease genes in regions identified by linkage. Improved techniques for high throughput identification and genotyping of polymorphism in open reading frames offer the possibility of extending this approach to understand and characterize the function and susceptibility of the human genome.

The base excision repair (BER) pathway is involved in the correction of DNA modifications that arise either spontaneously or from attack by endogenous or exogenous sources of exposure [4,5]. These modifications may arise spontaneously, from replication errors or through chemical modification by oxidation or alkylation. Anti-cancer agents and various environmental mutagens generate many of these types of lesions. BER involves the concerted effort of several repair proteins that recognize and excise specific DNA damages, working to replace the damaged moiety with "normal" DNA (Fig. 1) [6,7]. Typically, the first step in BER involves the removal of an inappropriate base by a DNA glycosylase. The abasic site that is produced by DNA glycosylase activity is subsequently recognized by an apurinic/apyrimidinic (AP) endonuclease (Ape1), which incises the phosphodiester backbone of DNA immediately 5′ to the lesion, leaving a strand break with a normal 3′-hydroxyl group and a non-conventional 5′-abasic residue. At this stage of the repair, mammalian BER can be directed into one of two sub-pathways depending on the ends of the substrate. The "Short-patch BER" pathway is preceeded with DNA polymerase β (Polβ) removing the 5′-abasic residue and filling in the single nucleotide gap. The alternative "long-patch" BER pathway entails the replacement of more than a single nucleotide (∼7–12 nucleotides), is PCNA-dependent (or stimulated) and requires FEN1 to excise the flap-like structure produced by DNA polymerase strand displacement (most frequently executed by DNA polymerase δ or ξ). In either scenario, DNA Ligase I or a complex of XRCC1 and Ligase III seals the nick and completes BER restoring DNA to its normal state.

Abbreviations: BER, base excision repair; Ape1, apurinic/apyrimidinicendonuclease.

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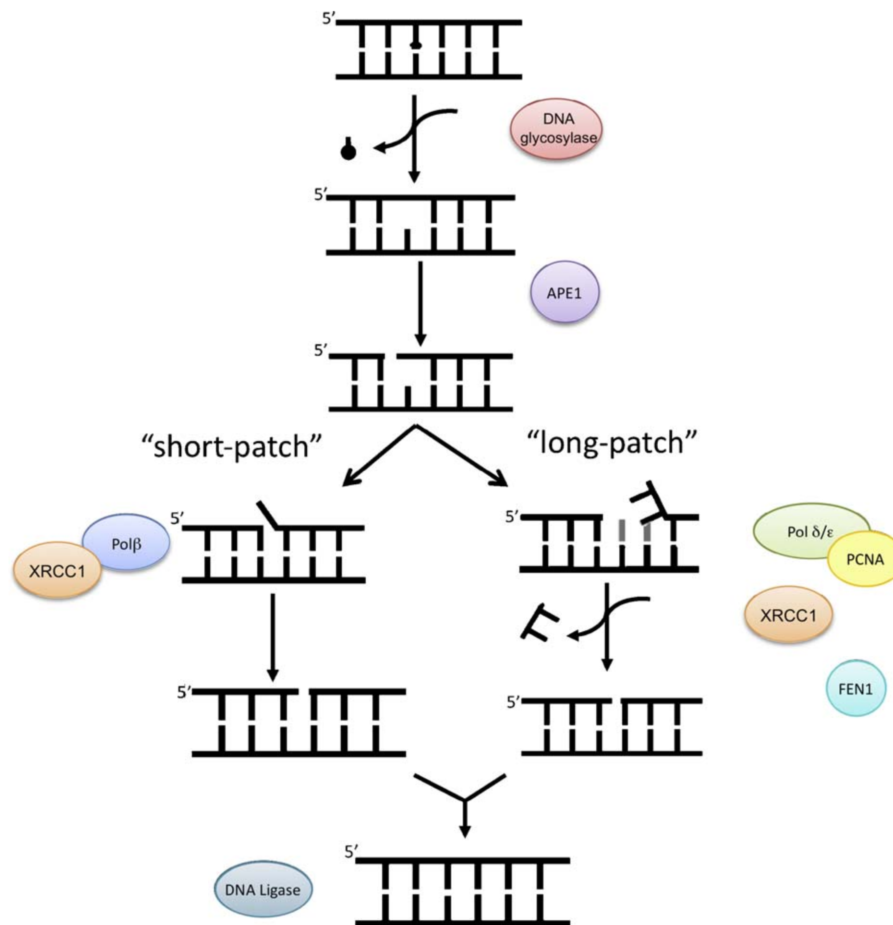

Fig. 1. Representation of the base excision repair pathway. In a highly co-ordinated fashion, damaged DNA in the form of oxidized or alkylated bases are recognized and removed by DNA glycosylase. The abasic site product is a substrate for AP endonuclease (Ape1), and is removed by incising the backbone 5′ to the lesion. Depending on various factors such as lesion type and cell cycle stage, the lesions are either repaired by "short-patch", where DNA Polβ removes the abasic residue and fills in the single nucleotide gap or "Long-patch" repair entails the replacement of 2–10 nucleotides by DNA δ/ε and requires PCNA and FEN1 proteins. XRCC1 has been shown to be an accessory protein for the sub-pathways. Finally, DNA ligase seals the nick and concludes the repair.

Given the known relationship of DNA repair to cancer and environmental exposures [3,4], the polymorphic variants identified have the potential to be population cancer risk factors because of the large number of individuals affected. Genes involved in DNA repair, such as those found in the BER pathway, are critical for protecting against mutations that lead to cancer and/or inherited genetic diseases [3,8–11]. Genes that are associated with an increased risk in sporadic cancer cases are referred to as "susceptibility" genes. Previous work to define the role of cancer susceptibility genes has often focused on variation in activity of carcinogen metabolizing enzymes with variant alleles that are associated with an increase in cancer risk [12,13]. The ability to measure DNA damage repair capacity *in vitro* has also provided insight into an individual's level of susceptibility [14–16]. The resequencing of DNA repair genes from several pathways have revealed variation in possible susceptibility genes [17,18], and several labs have demonstrated the feasibility of searching the public domain databases such as dbEST to identify single nucleotide polymorphisms (SNPs) [19–21]. To further molecular epidemiology studies that address the role of genetic variation of DNA repair genes in cancer susceptibility, we have screened the EST database to identify variation at the level of amino acid substitutions in the Ape1 gene. Single nucleotide polymorphisms resulting in coding errors (missense, in particular) were further examined using algorithms SIFT and PolyPhen to predict the impact of the amino acid substitution on enzyme structure [22–25]. These APE1 variants are candidates for future protein structure function studies as well as molecular epidemiology studies for understanding their role in disease susceptibility.

2. Materials and methods

2.1. Identification of variation within the Ape1 gene

To screen for amino acid substitutions in Ape1, we compared the amino acid sequence of Ape1 (accession #M92444) against the most recent EST database (build 130) using the tBlastn algorithm (http://www.ncbi.nlm.nih.gov/BLAST/) [26–28]. A total of 155 Ape1 or Ape1-related sequences were screened. Only those variants that were observed more than once were scored, as possible amino acid substitutions and the rest were not included in this study; nonsense (*) or ambiguous (B, Z, X, etc.) amino acid substitutions were also not considered for further analysis. Additional Ape1 amino acid variations were obtained from the SNP homepage at NCBI (http://www.ncbi.nlm.nih.gov/SNP/index.html). All scored variant DNA sequencing traces were verified by downloading the respective EST trace data from http://genome.wustl.edu/genomes and importing the traces in to the Genetic Annotation Initiative web server (http://www.chlc.org/gai/) for both amino acid substitution identification as well as SNP analysis.

2.2. Predicting impact of amino acid substitutions in the Ape1 variants

The possible impact of the amino acid substitutions in Ape1 variants were examined using PolyPhen and SIFT software [22–25]. The Ape1 amino acid sequence in GenBank accession #M92444 was used as wild type sequence. Solvent accessible surface areas of Ape1 residues were calculated by GETAREA [29] using the following crystal structures (PDB ID: 1E9N, 1DE8, 1DE9) [30,31]. Molecular models for the Ape1 variants were built using Pymol [32] to visualize the potential structural changes of the different Ape1 variants.

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