DNA polymerase β variant Ile260Met generates global gene expression changes related to cellular transformation

Katherine A. Donigan1,2, David Tuck3, Vince Schulz4 and Joann B. Sweasy1,2,*

1Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT 06520, USA, 2Department of Genetics, Yale University School of Medicine, New Haven, CT 06520, USA, 3Department of Pathology, Yale University School of Medicine, New Haven, CT 06520, USA and 4Department of Pediatrics, Yale University School of Medicine, New Haven, CT 06520, USA

*To whom correspondence should be addressed: Joann B. Sweasy, Ph.D.
Department of Pediatrics, Yale University School of Medicine, New Haven, CT 06520-8040, USA. Tel: +1 (203) 737 2626; Fax: +1 (203) 785 6309.
Email: joann.sweasy@yale.edu

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Maintenance of genomic stability is essential for cellular survival. The base excision repair (BER) pathway is critical for resolution of abasic sites and damaged bases, estimated to occur 20,000 times in cells daily. DNA polymerase β (Pol β) participates in BER by filling DNA gaps that result from excision of damaged bases. Approximately 30% of human tumours express Pol β variants, many of which have altered fidelity and activity in vitro and when expressed, induce cellular transformation. The prostate tumour variant Ile260Met transforms cells and is a sequence-context-dependent mutator. To test the hypothesis that mutations induced in vivo by Ile260Met lead to cellular transformation, we characterized the genome-wide expression profile of a clone expressing Ile260Met as compared with its non-induced counterpart. Using a 1.5-fold minimum cut-off with a false discovery rate (FDR) of <0.05, 912 genes exhibit altered expression. Microarray results were confirmed by quantitative real-time polymerase chain reaction (qRT-PCR) and revealed unique expression profiles in other clones. Gene Ontology (GO) clusters were analyzed using Ingenuity Pathways Analysis to identify altered gene networks and associated nodes. We determined three nodes of interest that exhibited dysfunctional regulation of downstream gene products without themselves having altered expression. One node, peroxisome proliferator-activated protein γ (PPARG), was sequenced and found to contain a coding region mutation in PPARG2 only in transformed cells.

Further analysis suggests that this mutation leads to dominant negative activity of PPARG2.

PPARG is a transcription factor implicated to have tumour suppressor function. This suggests that the PPARG2 mutant may have played a role in driving cellular transformation. We conclude that PPARG induces cellular transformation by a mutational mechanism.

Introduction

Human tumours exhibit genomic mutation levels much higher than the levels present in normal cells, and this increased mutation load cannot be accounted for by the somatic mutation rate alone. The mutator phenotype hypothesis, first put forth by Loeb and colleagues in 1974, proposes that the dramatic increase in mutagenesis observed in tumours may be attributed to DNA polymerase mutations that result in a reduction in polymerase fidelity (1). Pre-malignant cells with elevated levels of mutagenesis have an increased probability of acquiring mutations in genes that regulate vital cellular processes, including growth control, DNA damage response, apoptosis and cell–cell signalling. The role of mutagenesis in tumour development and progression is highlighted by studies published as part of the Cancer Genome Atlas (TCGA, http://tcga-portal.nci.nih.gov). Large-scale sequencing efforts targeting all predicted coding exons in breast and colon cancers have revealed that close to 10% of the ~18000 genes sequenced harboured a detectable mutation (2,3). In these studies, much heterogeneity between tumours was observed and no new commonly mutated genes were identified. Such high levels of heterogeneous mutagenesis identified in tumours are thought to be a driving force of tumour evolution and adaptability (4).

The base excision repair (BER) pathway functions to suppress mutagenesis and promote genomic stability by repairing oxidative and alkylation DNA damage. Such lesions are estimated to endogenously occur upwards of 20000 times per cell daily (5). DNA polymerase β (Pol β) is the primary polymerase involved in the BER pathway by both deoxynucleobisphosphate (dRP)-lyase and polymerase activities (6). Short-patch BER is initiated by lesion recognition and removal by a damage-specific DNA glycosylase. If the glycosylase is non-functional, the DNA backbone is then incised by apurinic/apyrimidinic (AP) endonuclease, resulting in a nicked DNA substrate with a 3’-hydroxyl and a 5’-dRP group. Bifunctional glycosylases also cleave the DNA backbone, and enzymes including AP endonuclease and polynucleotide kinase remodel the DNA ends. Pol β binds this substrate, removes the dRP group and fills in the gap in a template-directed manner. DNA ligase IIIα completes the repair by sealing the nicked DNA backbone.

The Pol β variant Ile260Met was first identified in a prostate tumour (7). Residue Ile260 is located in the hinge region of Pol β, a hydrophobic area found between the finger and palm subdomains. The hinge and specifically residue Ile260 are critical for polymerase fidelity (8–10). The variant Ile260Met has been previously characterized in vitro (11). Ile260Met is active in BER and exhibits biphasic burst kinetics similar to wild-type (WT) with a 3-fold reduction in rate. Ile260Met exhibits ~5-fold reduced affinity for DNA as compared with WT. In an in vitro forward mutation assay, Ile260Met increases overall error frequency ~3-fold and generates microsatellite expansions 8-fold more than WT. Single turnover kinetic analysis revealed that Ile260Met is a sequence-specific mutator; the efficiency of dNTP misincorporation opposite a templating base varies depending on the flanking DNA sequence. These data indicate that sequence contexts “at risk” for mutation by Ile260Met are distinct from WT Pol β.
The cellular effects of Ile260Met expression have also been evaluated using non-transformed, immortalized C127 mouse cells that have a WT Pol β background (12). Expression of exogenous Ile260Met in C127 cells results in focus formation, at varying rates across different clones. Transformation persists in cells expressing Ile260Met after shutting off expression of Ile260Met, indicating that a heritable change was induced in these cells, as transformation is not dependent on continuous Ile260Met expression. In contrast, expression of exogenous WT did not result in cellular transformation, even at high passage numbers. These results, taken together with the in vitro data, indicate that the variant Ile260Met has a functional phenotype related to tumorigenesis and/or progression. Given that Ile260Met is a subtle mutator, mutations introduced in growth-control genes may underlie the observed cellular transformation. In this study, we sought to characterize the global gene expression profile of C127 cells transformed by Ile260Met. We wanted to determine which genes, pathways and networks were altered as a result of Ile260Met expression. We also employed genome-wide comparative genomic hybridization (CGH) to determine whether expression level changes were due to copy number variation (CNV).

Materials and methods

Cell lines and cell culture

C127 cells (ATCC) are a non-transformed immortalized cell line derived from a mammary tumour of an RIII mouse (13). The C127 clonal cell line expressing the Pol β variant Ile260Met, termed Ile260Met clone 2, used in this study was fully characterized and is described in our previous study (12). Briefly, to generate inducible, stable clones the parental C127 cell line was infected with retrovirus encoding the Ile260Met Pol β variant under the control of an inducible promoter (Tet off system) with hygromycin selection. Stable clones were selected with hygromycin and expanded to generate cell clonal lines. Exogenous expression was evaluated by western blot and only clones exhibiting an approximate 1:1 ratio of endogenous to exogenous Pol β were selected for further analysis. Ile260Met clone 2 was among six Ile260Met-expressing clones including clones 5 and 8, and was observed to induce cellular transformation only when expression was induced. Foci accumulate at varying rates in different clones, but all clones eventually produce foci too numerous to be counted. In contrast, C127 clones harbouring exogenous WT Pol β do not form significant numbers of foci under inducing or non-inducing conditions. CV-1 cells were a gift of Dr. Zhog Yun, Yale University.

Total RNA isolation

C127-Ile260Met clones 2, 5 and 8 were passed in the presence or absence of Tet and split into two T75 flasks per condition to generate biological replicates. Cells were grown to ~80% confluence. Total RNA was extracted using TRIzol and split into two T75 flasks per condition to generate biological replicates. C127-Ile260Met clones 2, 5 and 8 were passaged in the presence or absence of inducible promoter (Tet off system) with hygromycin selection. Stable clones harbouring exogenous WT Pol β were selected using non-transformed, immortalized C127 mouse cells as having altered expression levels were selected for quantification by complementary DNA (cDNA) was synthesized from 10 ng RNA using the First Strand cDNA Synthesis Kit with random hexamer and oligo-p(DT)18 primers according to the manufacturer’s instructions (Roche). For each qRT-PCR reaction, 5 μl of cDNA was used with the FastStart SYBR Green Master Mix with Rox as a reference dye (Roche). Primer pairs specific to each gene transcript of interest were designed using PrimerExpress software (Applied Biosystems) and synthesized by the Keck Oligonucleotide Synthesis Facility at the Yale School of Medicine (supplementary Table 1, available at Mutagenesis Online). All amplification reactions used primers of 18–22 nucleotides to generate target amplicons of ~60 bp. mRNA levels of transcripts of interest were compared with the control HPRT1 to normalize cDNA levels. Each qRT-PCR reaction was performed in a final volume of 50 μl in triplicate using the Stratagene MX3000 instrument with the following reaction conditions: activation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 30 s, primer annealing at 60°C for 1 min, extension at 72°C for 30 s. Target transcript levels were quantified using the comparative C method to determine relative fold changes (16).

Genome-wide CGH

To determine whether any gene expression alterations result from CNV, we used gDNA from Ile260Met clone 2 grown under inducing or non-inducing conditions for genome-wide CGH experiments. The Mouse CGH 3×720K whole-genome tiling array (Nimblegen) was used and hybridization was performed as a fee for service by the Yale Center for Genome Analysis at the Keck Center for Biotechnology at Yale University. Copy number data were processed using the R, version 2.9.0 statistical computing environment. The data for the two colour channels for the array sections was log transformed and data were then normalized together using the normalize oligo function (17). Log ratios of induced and non-induced data were generated and these data were segmented using the Faseg package to identify regions of copy number change (18). The segmented and normalized data were visualized using IGB software (19).
PCR amplification and sequencing of nodes of interest

Three nodes of interest, peroxisome proliferator-activated receptor γ (PPARG; NM_011146), transformation protein 53 (TP53; NM_011640) and platelet-derived growth factor-β (PDGFβ; NM_011057) were identified using IPA network analysis. Nodes were directly sequenced from gDNA isolated from Ile260Met clone 2. PPARG was also directly sequenced from gDNA isolated from Ile260Met clones 5 and 8. All exons were amplified using the primer sequences in supplementary Table 2 (available at Mutagenesis Online) and the following PCR conditions: activation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, primer annealing at 57°C for 1 min and extension at 72°C for 30 s, followed by a 10-min hold at 72°C after the completion of 35 cycles. PCR products were evaluated by electrophoresis on a 2% agarose gel and sequenced at the Keck DNA Sequencing Facility at the Yale School of Medicine.

Luciferase assay for PPARG2 function

The peroxisome proliferator response element-luciferase (PPRE-luc) vector was a gift from Alfred Bothwell, Yale University. The pBABE-PPARG2 (mouse) vector was a gift from Dr Zhong Yun, Yale University. Standard methods of site-directed mutagenesis (Stratagene) were employed according to the manufacturer’s directions to mutate Pro-495 to Leu and Leu-21 to Met. In our studies, each of the PPARG2 constructs contains the Pro495Ala alteration in order to eliminate the effects of endogenous ligands on the assay and allow us to assess the effect of the Leu21Met mutation, present in the domain responsible for ligand-independent activity (20). The Renilla plasmid was a gift from Dr Daniel DiMaio, Yale University. Approximately 4×10⁴ CV-1 cells were seeded into each well of 24-well microtitre plates and allowed to attach overnight as described elsewhere (20). The cells were transfected in triplicates using lipofectamine with 2 ng of the Renilla plasmid, 300 ng of PPRE-luc and 150 ng of the pBABE-PPARG2-WT or pBABE-PPARG2-Leu21Met plasmids. After 5–6 h, the media was replaced with DMEM supplemented with 10% FBS-10. The next day, the media was replaced with serum-free media with or without 5 µM Troglitazone, an activating ligand of PPARG. On the following day, the cells were lysed and luc was quantified using a luminometer. Light units from luc were normalized to light units from Renilla to obtain relative light units (RLU). The experiment was repeated three times and data are reported as the average of triplicates from a representative experiment. Data were analyzed using one-way analysis of variance with Bonferroni’s multiple comparison test using GraphPad Prism (V 5.0) software.

Results

Cells expressing Ile260Met exhibit an altered global gene expression profile

Ile260Met expression has been previously shown to induce cellular transformation in C127 cells as measured by focus formation and anchorage-independent growth (12). This expression is inducible in a Tet off system, allowing us to compare induced and non-induced versions of the same Ile260Met clone. When cells express Ile260Met (grown in the absence of Tet), cellular transformation is observed and when Ile260Met is not expressed (grown in the presence of Tet) cellular transformation is not observed. Expression of Ile260Met is extinguished by supplementing the media with Tet; however, there is no observed decrease in cellular transformation, suggesting that transformation results from mutagenesis induced by Ile260Met when it is expressed in the cells (12).

To identify pathways that may have been altered during cellular transformation induced by Ile260Met, we profiled 34,000 genes, comparing gene expression in transformed versus non-transformed C127-Ile260Met clone 2. We identified a large number of genes in our raw data set with altered expression between transformed and non-transformed cells (Table 1). Applying an FDR cut-off (<0.05) to our raw data set decreased the number of altered genes by ~40–65% depending on the minimum fold change cut-off applied. The data set with a minimum fold change of 1.5 and FDR <0.05 (912 genes) was selected for further analysis. The distribution of fold changes is indicated in Figure 1.

Although most changes are within the 3-fold range, we observed much larger fold changes in some genes. The top 10 most up- and down-regulated genes are indicated in Table II. Altered expression of many of these genes is linked to cancer etiology. GSTA1/2, up-regulated 11- to 20-fold in cells transformed by Ile260Met, are involved in detoxification of carcinogens and their over-expression has been observed in tumours and implicated in resistance to chemotherapeutic agents (21). KRT14, also up-regulated in our cells, codes for a member of the keratin family that contributes to cytoskeleton formation and basement membrane adhesion. KRT14 has been reported to be significantly over-expressed in metastatic primary breast tumours, and is thought to contribute to metastasis dissemination through involvement in extracellular matrix stability (22). We also found the oncogenic transcription factor MYCN to be up-regulated in cells transformed by Ile260Met. MYCN amplification, first characterized in aggressive neuroblastoma tumours, has been shown to result in cell cycle deregulation, angiogenesis stimulation and increased proliferation (23). Enrichment of these genes in cells would be expected to promote tumorigenesis.

In addition to genes that were significantly up-regulated in cells transformed by Ile260Met, we identified many genes that were significantly down-regulated. CD36, the most down-regulated gene we identified, is a cell surface receptor that binds thrombospondin-1 to inhibit angiogenesis (24). G0S2, down-regulated 8-fold in our cells, is mitochondrial pro-apoptotic factor that is epigenetically silenced in tumours and cancer cell lines (25). We also observed a nearly 5-fold down-regulation
of MMP19, a matrix metalloproteinase. MMP19 exerts anti-angiogenic effects by inhibiting vascular endothelial growth factor production, and reduces tumour growth in nude mice (26). Cells transformed by Ile260Met exhibit down-regulation of genes that guard against tumour development and progression.

Seven genes altered in clone 2 are differentially expressed in other clones

Quantitative real-time PCR analysis (qRT-PCR) of seven genes was performed to validate our microarray results. The seven genes selected include the most up- and down-regulated genes and genes altered at the lower end of our threshold cut-off to confirm modest expression level changes as real. Intermediately altered genes (i.e. POLB and JUN) were randomly chosen. Transcript-level fold changes obtained from microarray analysis are comparable with those obtained from qRT-PCR (supplementary Figure 1, available at Mutagenesis Online). We then decided to evaluate the expression levels of these seven genes by qRT-PCR in two additional Ile260Met clones. Similar to clone 2, clones 5 and 8 were previously shown to form large numbers of foci when Ile260Met expression is induced (12). Each of these three clones was observed to form foci at different rates, indicating that the mechanism underlying transformation may be different in each clone. Expression levels of the seven genes evaluated by qRT-PCR vary from clone to clone (Figure 2). Clone 8 exhibits modest up-regulation or no change for all genes evaluated. Clone 5 exhibits down-regulation for five of the seven genes, including the genes most up-regulated in clone 2. Therefore, the expression profiles of each clone are probably different from each other.

Gene expression alterations do not result from genomic CNV

To determine whether, if any, gene expression alterations were the direct result of genomic CNV, we performed genome-wide CGH using gDNA isolated from Ile260Met clone 2 grown under both inducing and non-inducing conditions. The Nimblegen Mouse CGH whole-genome array used here contains 720,000 probes on each array with median probe spacing of 3537 bp. We did not observe any genomic region exhibiting CNV between inducing and non-inducing conditions. Taken together, our data are consistent with the interpretation that Ile260Met generates global gene expression alterations by a stochastic, mutagenic mechanism that is independent of CNV.

Global gene expression changes resulting from Ile260Met expression are related to cancer etiology

Genes with a minimum fold change of 1.5 and an FDR <0.05 were included in IPA pathway, disease and network analysis defined by the IPA database. Cancer was the most significant disease association for this data set, and included 211 genes with a P value range from $1.87 \times 10^{-11}$ to $7.39 \times 10^{-3}$. The most significant molecular and cellular functions associated with Ile260Met expression are indicated in Table III. These functions are related to tumorigenesis and are probably related to the transformation phenotype observed when Ile260Met is expressed.

The data set used for IPA analysis was then used for GO analysis. We identified 18 GO categories with Fisher P values $<0.05$ (supplementary Table 3, available at Mutagenesis Online) and separately analyzed gene networks for each of these clusters using IPA. Gene network analysis permits the examination of relationships between altered genes or gene products that are related in some way to a common gene but may not themselves be within the same pathway. The common genes that are central to a network and are connected to multiple genes across different pathways are referred to as “node genes”. We identified three nodes of interest across three different GO categories (Figure 3 and supplementary Figures 2 and 3, available at Mutagenesis Online). These three nodes, PPARG, TP53 and PDGFB, are not themselves altered at the transcript level. The genes or gene products affected by these node genes exhibit altered gene expression, as many of these affected genes are altered in the opposite direction from their relationship to the node. The transcription factor PPARG has
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been shown to up-regulate CD36 expression through binding of a peroxisome proliferator response element (PPRE) within the gene’s promoter (27,28). In the gene network generated from the GO:0008135 cluster (nucleic acid binding), PPARG is a node gene connected to CD36 (Figure 3). In cells transformed by Ile260Met, CD36 is down-regulated 15-fold at the transcript level. PPARG has been shown to up-regulate pyruvate carboxylase (PC), a gene involved in gluconeogenesis and insulin secretion (29). Cell death-inducing DNA fragmentation factor-α-like effector C (CIDEC) is an adipocyte lipid droplet protein that is a member of the pro-apoptotic CIDE family (30). The CIDEC gene also contains a PPRE and has been shown to be up-regulated by PPARG (31). Similar to PC and CD36, CIDEC is down-regulated in cells transformed by Ile260Met.

Although not present in this gene network, the second most down-regulated gene G0S2 contains a PPRE and has been shown to be directly up-regulated by PPARG (32). These observations led us hypothesize that PPARG may contain a functional mutation, as it was unaltered at the transcript level. Similar trends were observed for several genes connected to the nodes TP53 and PDGFB (supplementary Figures 2 and 3, available at Mutagenesis Online). Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 1 (APOBEC1) is a cytidine deaminase involved in mRNA editing (33) and is up-regulated by TP53 (34). In cells transformed by Ile260Met, APOBEC1 is down-regulated as compared with their non-transformed counterpart. Poliovirus receptor-related 3 (PVRL3, also known as Nectin3) is implicated to play a role

Fig. 3. Gene network featuring dysregulated PPARG node. The nodes within this network are TNF, ARNT2, ANGPT2 and PPARG. The node PPARG is functionally related to genes connected by arrows and the reported effects on gene regulation are indicated by the presence of a red plus symbol (up-regulation) and a green minus symbol (down-regulation). The IPA database indicates that the relationships between the node PPARG and CD36, CIDEC, PPIC and PC should result in up-regulation of these genes. Down-regulation of each of these four genes is observed in cells expressing Ile260Met, implying potential dysregulation by PPARG. The node TNF does not exhibit such dysregulation. For example, ALOX15B and SAMD4A are up-regulated by TNF and GPD1, CIDEC and UCP2 are down-regulated by TNF according to the IPA database, and all of these molecules are correspondingly up or down-regulated in cells expressing Ile260Met. This may indicate an increase in TNF activity in these cells, but does not indicate TNF dysfunction.

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The formation of cell–cell junctions (35), and according to the IPA database, it is negatively regulated by TP53. We found PVRL3 to be up-regulated in cells transformed by Ile260Met. The superoxide-generating oxidase MOX1 is up-regulated by PDGFB (36) and was found to be down-regulated in Ile260Met-transformed cells. The IPA database also indicated that PDGFB positively regulates microsomal glutathione S-transferase 1/2, which was down-regulated in cells transformed by Ile260Met.

We then sought to determine whether these nodes of interest harboured genomic mutations that could affect function. gDNA was isolated from cells that had been passaged in the absence (transformed) or presence (non-transformed) of Tet. Each individual exon for the central node gene was then sequenced from gDNA as described in Materials and methods. We identified a single, heterozygous coding region mutation in the transformed cells: C to A at the 106th residue of exon 1 of isoform PPARG2 (supplementary Figure 4, available at Mutagenesis Online). PPARG2 is a splice variant of the PPARG gene, and differs from PPARG1 only by an additional 30 amino acids at the N-terminus (37). The genomic mutation identified here results in the non-synonymous amino acid substitution Leu21Met. This mutation was not present in clone 2 grown under non-inducing conditions (the non-transformed counterpart). It was also absent from transformed clones 5 and 8 grown under inducing conditions. These results indicate that this PPARG2 mutation is unique to transformed clone 2.

The Leu21Met PPARG2 alteration results in decreased PPARG activity

To determine whether PPARG2 harbouring the Leu21Met alteration results in aberrant PPARG activity, we assessed the ability of this variant to bind to the PPRE and activate transcription of Luc, as described in (20) and above in Materials and methods. As shown in Figure 4, the cells transfected with PPRE-luc alone exhibit an increase in luciferase when Troglitazone is added due to the function of endogenous PPARG that retains ligand-binding activity. In the absence of Troglitazone, these cells have a modest level of luciferase, attributable to the ligand-independent PPARG activity and/or low levels of endogenous ligand. When exogenous WT PPARG (impaired for ligand binding by the Pro495Leu (495) mutation) is added, a similar luciferase response is seen both in the presence and absence of Troglitazone. There is no increase in luc response as compared with the PPRE-luc control; therefore the luc activity seen is attributable to endogenous PPARG. WT/495 does not interfere with endogenous PPARG activity. Conversely, cells expressing Leu21Met/495 exhibit significantly reduced luc levels as compared with the PPRE-luc control and the WT/495 cells, both in the presence and absence of Troglitazone. These results indicate that Leu21Met is functionally impaired and acts in a dominant negative manner to inhibit the activities (ligand-dependent and independent) of the WT endogenous PPARG. These results suggest that the Leu21Met variant could be responsible for aberrant transcriptional regulation, which could lead to cellular transformation.

Discussion

Our previous work with the tumour-associated Pol β variant Ile260Met led us to hypothesize that expression of Ile260Met results in mutations in growth-control genes, resulting in the cellular transformation phenotypes we observed (11,12). The goal of this study was to determine whether Ile260Met expression in cells was sufficient to generate mutations that resulted in global gene expression changes. Transformed cells exhibited expression profiles that were different from non-transformed cells of the same clone, and this was confirmed using qRT-PCR. In clone 2 cells expressing Ile260Met, we demonstrated that the expression of 912 genes was altered by a minimum of 1.5-fold at the transcript level, with an FDR <0.05. Importantly, genes identified as differentially expressed in clone 2 were not altered in the other clones analyzed by qRT-PCR. Therefore, expression of a single POLB tumour-associated repair variant, Ile260Met, probably in combination with selection for rapid growth, leads to massive changes in global gene expression. We suggest that Ile260Met induces mutations during the gap-filling step of BER and that certain alterations are selected during cellular growth. This suggestion is supported by the fact that Ile260Met...
is a mutator POLB variant that participates in BER (11). We have shown that a single mutation in the PPARG2 nodal gene, induced by Ile260Met in clone 2, has a phenotype that could result in aberrant transcriptional regulation and lead to cellular transformation. This single mutation was identified only in the transformed cells from clone 2. We sequenced this genomic region in the transformed cells from clones 5 and 8 as well and did not find this mutation (data not shown). These results are concordant with the qRT-PCR profiling performed on a subset of genes. Each clone had a unique expression profile for the genes we tested, indicating that the mutations present at the DNA level are likely to be different between clones as well. The isolation of a PPARG2 mutation in transformed cells of clone 2 without further experimental selection implies a biological selection for this particular mutation. In earlier passages of this line, one or a few cells may have obtained the mutation and received a growth advantage over other cells, taking over the population. Alternatively, many cells in the population could have received identical hits in this gene at different times but given the stochastic nature of mutagenesis, we consider this to be unlikely.

Because many of the altered genes we identified are associated with carcinogenesis, according to GO and IPA analysis, our data are consistent with the interpretation that cellular transformation results from aberrant gene expression. Ile260Met was identified in a prostate tumour and has the opportunity to drive rapid evolution of the tumour by mechanisms similar to those observed in our study.

Each transformed clone of Ile260Met studied here does not exhibit similar gene expression profiles for the genes we analyzed. This suggests that expression of Ile260Met induces different mutations in each of the clones, as would be expected, given that DNA damage-induced mutagenesis is not a directed process but is essentially random in nature. Therefore, our results point to the possibility that expression of DNA repair variants in tumours has the potential to drive rapid evolution of tumour cells in response to the microenvironment.

Sequence-context specificity of Ile260Met may facilitate fitness

In an in vitro forward mutation assay, Ile260Met does not greatly increase the overall mutation frequency (11). Instead, it is characterized as a sequence context-dependent mutator polymerase, because it increases the mutation frequency within certain DNA contexts. These contexts would be at elevated risk for mutagenesis as compared with cells expressing WT Pol β. In fact, the PPARG mutation coding the Leu21Met PPARG protein is C to A, and Ile260Met has a greater propensity than WT Pol β to insert dTTP opposite template C. A sequence-context-dependent mutator may not challenge cellular fitness to the same degree as a highly mutagenic Pol β variant, which would be more likely to be selected against in the tumour population, as the introduction of many mutations over a relatively shorter period of time would be less compatible with tumour fitness. As a subtle mutator, Ile260Met would be expected to increase mutagenesis only when presented with specific sequence contexts during BER. Such events would be rarer than generalized mutagenesis, and thus more likely to be compatible with tumour fitness.

Cellular transformation may result from alteration of PPARG

IPA analysis of the 912 genes identified by expression profiling of Ile260Met clone 2 revealed that 211 of them were implicated in cancer, the most significant disease associated with our data set. PPARG is a transcription factor that is activated by certain fatty acids and thiazolidinediones (38). PPARG forms a heterodimer with RXRα and binds PPREs to activate the expression of numerous genes by recruiting co-activators and displacing co-repressors (29). The isoform PPARG2 has been found to be primarily expressed in adipose tissue, where it is implicated in the regulation of adipogenic differentiation (39,40). We performed qRT-PCR using primers specific for isoform PPARG2 and found that this isoform is expressed in both transformed and non-transformed clone 2, and this expression was approximately equal between the conditions (data not shown).

Residue 21 of PPARG2 is located in the protein’s N-terminal domain (NTD). Interestingly, the NTD has been shown to regulate the binding of PPARG2 with its co-activators, and truncation of the NTD results in a dominant negative effect that reduces transcription from a PPRE (20). The NTD of PPARG2 has also been shown to have ligand-independent activation 5- to 6-fold greater than PPARG1 (41). Therefore, mutations resulting in loss-of-function within the NTD of PPARG2 would be expected to have a greater effect on its ligand-independent activation as compared with PPARG1. Here, we show that this is probably the case, because the Leu21Met mutant protein is deficient in activating transcription from a PPRE when as compared with WT, as would be predicted from our microarray experiments. Strikingly, when co-expressed with endogenous WT, Leu21Met also inhibits the ability of WT to activate transcription.

WT PPARG activation has been shown to inhibit anchorage-independent growth in human colorectal cancer cells and trigger G1 arrest (42). Ameshima and colleagues have demonstrated that the expression of a dominant negative PPARG variant in ECV304 cells renders them hyperproliferative, angiogenic as evidenced by tube formation in matrigel, and resistant to apoptosis (43). The results indicate that WT PPARG exhibits anti-proliferative effects and may help suppress tumorigenesis. We have previously reported that cells expressing Ile260Met exhibit phenotypes related to transformation, including anchorage-independent growth. The work presented here indicates that a C127 clone induced to express Ile260Met also expresses the PPARG2 variant Leu21Met, resulting in misregulation of several genes associated with cancer. We suggest that the aberrant gene expression that results from the Leu21Met PPARG2 variant contributes to cellular transformation via aberrant transcriptional regulation. We note that several other genes are up- or down-regulated in the Ile260Met-transformed cells, and it is unlikely that all of these genes are regulated by PPARG. Thus, alteration of non-PPARG-regulated genes could also lead to cellular transformation. Finally, combinations of misregulated genes may be critical for the induction of cellular transformation.

Taken together, our results show that mutagenesis is one mechanism underlying Ile260Met-mediated cellular transformation. Cells expressing Ile260Met exhibit a significantly altered global gene expression profile that is enriched for genes related to cancer etiology. Our CGH data indicate that changes in DNA copy number do not contribute to expression variation in our cells expressing Ile260Met. Through gene network analysis, we were able to isolate a genomic mutation resulting in a non-synonymous amino acid substitution in a transcription factor associated with growth control, PPARG. Our interpretation that Ile260Met-related transformation is caused by mutagenesis, a stochastic process, is further supported by the dynamic rate at which other Ile260Met-expressing clones transformed,
as well as the differences in gene expression for the seven genes evaluated by qRT-PCR.

Small-scale studies indicate that Pol β variants are present in 30% of human tumours, and are found in multiple tumour types (44). In the work presented here, we have observed the ability of a single Pol β variant to cause dramatic changes in global gene expression related to the induction of cellular transformation (12,45). The isolation of functional Pol β variants from human tumours has significant implications for tumorigenesis and/or progression.

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Supplementary data

Supplementary Tables 1–3 and Figures 1–4 are available at Mutagenesis Online.

Conflict of interest statement: None declared.

References

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