

# ZNF198 protein, involved in rearrangement in myeloproliferative disease, forms complexes with the DNA repair-associated HHR6A/6B and RAD18 proteins

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**A highly specific t(8;13)(p11;q12) translocation has been consistently identified in bone marrow cells from patients with an atypical myeloproliferative disease that is associated with peripheral blood eosinophilia and T- or B-cell leukemias. In all patients analysed to date, the translocation event results in a chimeric gene in which the atypical zinc-finger domain of ZNF198 is fused to the N-terminal end of the catalytic domain of the FGFR1 receptor tyrosine kinase. To understand more about the consequences of this rearrangement we have investigated the normal function of the ZNF198 gene. Using yeast two-hybrid analysis we identified HHR6 as a protein binding partner and confirmed this using immunoprecipitation studies. The ZNF198/FGFR1 fusion protein also binds to HHR6. We demonstrate here that the human RAD18 is also present in the ZNF198/HHR6 protein complex, although it does not coimmunoprecipitate with the fusion kinase. Cells expressing the fusion kinase gene show a marked increased sensitivity to UVB irradiation, suggesting that it acts in a dominant-negative way to affect DNA repair. These observations support the idea that ZNF198, through its interaction with HHR6 and RAD18, may be involved in the DNA repair process.**

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## Introduction

The characterization of chromosomal translocations in specific types of leukemia has consistently led to the isolation of fusion genes important for the leukemogenesis process. The normal counterparts of these genes, however, have been shown to have diverse functions. An atypical chronic form of myeloproliferative disease (MPD) was described some years ago (Abruzzo *et al.*, 1992), which is associated with T-cell leukemia/lymphoma and peripheral blood eosinophilia. Cytogenetic analysis of

bone marrow aspirates from these patients showed a consistent reciprocal chromosome translocation t(8;13)(p11;q12). In some cases this rearrangement was the only cytogenetic abnormality. In our initial studies we identified the position of the translocation breakpoints using FISH (Kempinski *et al.*, 1995) and then used somatic cell hybrids to clearly define the position of the breakpoints on both chromosomes (Still *et al.*, 1997; Chernova *et al.*, 1998). The 8p11 translocation breakpoint was subsequently shown to interrupt the FGFR1 gene (Smedley *et al.*, 1998; Still and Cowell, 1998; Xiao *et al.*, 1998; Popovici *et al.*, 1999) and the chromosome breakpoint in 13q12 was shown by several groups to involve a zinc-finger-containing gene, ZNF198 (also called RAMP8 and FIM). Despite some discrepancies in early reports (Smedley *et al.*, 1998; Xiao *et al.*, 1998) the full-length structure of the ZNF198 gene and the nature of the fusion gene were finally resolved (Still and Cowell, 1998). This demonstrated that the chimeric gene resulted from the in-frame fusion of the ZNF198 zinc-finger motif with the tyrosine kinase domain of FGFR1. It has been suggested (Smedley *et al.*, 1998; Baumann *et al.*, 2003) that the ZNF198 zinc-finger motif mediates dimerization of the fusion protein, resulting in constitutive activation of the tyrosine kinase, leading to tumorigenesis.

ZNF198 is a widely expressed gene and is predicted to encode a 1377 amino-acid protein with a molecular mass of 150 kDa. The most prominent features of ZNF198 are: five zinc-fingers, a proline-rich region and a C-terminal acidic domain containing a putative nuclear localization signal. The zinc-fingers in ZNF198 are of the type Cys-X<sub>2</sub>-Cys-X<sub>19–22</sub>-Cys-X<sub>3</sub>-Cys-X<sub>13–19</sub>-Cys-X<sub>2</sub>-Cys-X<sub>19–25</sub>-Cys-X<sub>3</sub>-Cys, which are generally predicted to have a role in protein–protein interactions (Mackay and Crossley, 1998), unlike the usual members of the Kruppel-like family which are DNA-binding transcription factors. To gain a better understanding of the function of ZNF198, we used the yeast two-hybrid assay to identify potential interacting proteins. Using this approach we have established that ZNF198 binds to the human homologues of the yeast RAD6 protein, HHR6A and HHR6B (Koken *et al.*, 1991). RAD6 is a ubiquitin-conjugating DNA repair enzyme (Jentsch *et al.*, 1987) associated with postreplication repair. The

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interaction between ZNF198 and HHR6 was confirmed using immunoprecipitation (IP) using GFP-tagged proteins and Western blotting. It has been shown recently that the RAD18 protein forms a complex with both human and yeast RAD6 (Tateishi *et al.*, 2000; Xin *et al.*, 2000). Western blot analysis revealed that RAD18 was also present in the IP complex containing ZNF198 but not the ZNF198/FGFR1 fusion protein. Furthermore, overexpression of the fusion kinase gene affects cell survival following UV irradiation. These observations provide the first evidence for a function of ZNF198, which is involved in a protein complex one of whose functions is to be related to DNA repair.

## Materials and methods

### *Yeast two-hybrid analysis*

The ZNF198 open-reading frame (ORF) was cloned into the multiple cloning site of the pAS2.1 vector to generate pASZNF198. This construct fuses the ZNF198 ORF, in-frame, to a GAL4 DNA-binding domain under the control of the alcohol dehydrogenase (ADH) promoter. This construct was used to transform the TRP<sup>-</sup>, LEU<sup>-</sup>, HIS3<sup>-</sup> CG1945 yeast strain. Transformants were selected on SD medium lacking only tryptophan because the pAS2.1 vector carries a functional TRP gene. A single pure colony containing pAS-ZNF198 served as the host for transfection with DNA prepared from the commercially available 'Matchmaker' fetal brain cDNA library (BD Biosciences Clontech, Palo Alto, CA, USA). The cDNA library was transformed into the pAS-ZNF198/CG1945 cells using the lithium acetate method according to the manufacturer's protocol. At the same time, the ZNF198-GAL4 DNA-binding domain was expressed in yeast host cells carrying the pGAD10 vector in the absence of cloned cDNAs, which demonstrated that the ZNF198 fusion product has no intrinsic transactivation activity. Positive growth selection was accomplished on SD medium lacking tryptophan, leucine and histidine in the presence of 10 mM 3-aminotriazole. Complementation of the HIS<sup>-</sup> phenotype of CG1945 occurs by the GAL4-dependent expression of the HIS3 reporter gene as a result of the generation of a functional GAL4 transcription factor binding to the GAL4 response elements (UAS<sub>G</sub>). This will occur by the interaction between the ZNF198 fusion protein and unknown interacting partners. His<sup>+</sup> colonies were transferred to gridded nylon filters and assayed for the  $\beta$ -galactosidase activity in the presence of X-gal at 30°C, and blue colonies selected for further analysis. This dual selection for expression of HIS3 and LacZ, acts to reduce false positive clones.

To further confirm that there was a true interaction of proteins within the colonies selected, we subjected them to selective mating assays (Harper *et al.*, 1993). The pAS2.1 vector contains the CYH2 gene, which confers cycloheximide sensitivity to the otherwise cycloheximide-resistant yeast host. Plating putative positive clones on SD media containing cycloheximide, in the absence of leucine, selects for loss of the DNA-binding domain construct pASZNF198, while retaining the pGAD10 construct carrying the putative interacting partner. These clones can then be mated with Y187, a yeast of opposite mating type that express the ZNF198-fusion construct or unrelated proteins as GAL4 DNA-binding domain fusions, and diploids assayed for  $\beta$ -galactosidase activity. Only those clones, which express  $\beta$ -galactosidase specifically with the pASZNF198 construct, were considered for further analysis.

The plasmid cDNA clones were recovered from total yeast DNA preparations and transferred into chemically competent DH5 $\alpha$  (Invitrogen, Carlsbad, CA, USA). These clones were then sequenced by the RPCI Biopolymer Core Facility.

### *Generation of a peptide antibody against ZNF198*

To predict potential antigenic sites within the ZNF198 protein, we used the Peptide software available at NCBI and identified two candidate, potentially antigenic, amino-acid sequences: (1) <sub>97</sub>SKITPSSKELASQKG<sub>111</sub> and (2) <sub>216</sub>HVTSLQNTNLGDVSN<sub>231</sub>. Several nascent synthetic peptide chains were synthesized on a multifunctional small synthetic polylysine core attached to a solid-phase resin (Tam, 1988). The resulting multimeric peptide antigen was then separated from the resin and used directly for immunization of rabbits without the need for further purification (Rockland, Gilbertsville, PA, USA). This approach resulted in the generation of a relatively specific polyclonal antibody against the N-terminal end of the ZNF 198 gene containing the zinc-finger region.

### *Vectors and plasmid construction*

The ZNF198/FGFR1 fusion gene was amplified by PCR from leukemic cells expressing the fusion gene. The PCR product was then cloned into the pEGFP-C2 vector as an N-terminal GFP fusion protein. The ZNF198 gene was amplified from a fetal bone marrow cDNA library and similarly cloned into pEGFP-C2 vector. All constructs were sequenced to confirm the correct orientation and sequence before being used for transfection studies.

### *Cell culture and transfection studies*

Human embryonic kidney (HEK) 293 cells were maintained in DMEM with 10% FBS in 5% CO<sub>2</sub>. 293 cells were used to establish cells stably expressing ZNF198 and the ZNF198-FGFR1 fusion kinase independently. Green cells were identified by fluorescence microscopy and colonies, selected in culture medium containing 500  $\mu$ g/ml of G418, were isolated using ring cloning. Specific gene expression was confirmed using RT-PCR and protein expression was confirmed by Western blotting using an anti-GFP polyclonal antibody (Clontech) as well as the ZNF198 antipeptide polyclonal antibody described above.

### *Immunoprecipitations and Western Blotting*

Cells were grown to 80% confluency and then washed twice with PBS. Lysis was achieved in RIPA buffer (50 mM Tris containing 150 mM NaCl 0.1% SDS 1% Triton X-100 1% sodium deoxycholate pH 7.2) with protease inhibitor cocktail (Sigma Aldrich, MO, USA) on ice for 10 min. Following centrifugation at 14 000 r.p.m. for 20 min, the supernatant was collected and used for IP. Lysates were precleared using sepharose-coupled rabbit IgG, and then the supernatant was treated with 3  $\mu$ l of anti-GFP rabbit polyclonal serum (Clontech) per 500  $\mu$ l of the lysates. The immune complexes were recovered with Protein A-sepharose. The sepharose beads were washed five times with PBS and then proteins were eluted by incubation in SDS sample buffer at 100°C for 3 min and aliquots were subjected to SDS-PAGE and Western blot analysis. Antibodies used for Western blot analysis are HHR6A&B, HHR6B (kind gift of Dr JH Hoeijmakers, Netherlands) and HRAD18 (Imgenex, CA, USA) antibodies.

### Cell survival studies following UV irradiation

Cells were grown to 70% confluency in 60 mm Petri dishes. The medium was then removed and the cells washed in PBS. Four replicates of the cells were then exposed to various doses of UVB irradiation (280–320 nm) while still in PBS. The PBS was then removed and DMEM plus 10% FCS was added. Cells were incubated for 72 h and cell death was measured using the Trypan blue exclusion method.

## Results

### Yeast two-hybrid analysis

The structure of the ZNF198 protein provides few clues about its specific function. The zinc-finger region, however, is suggestive of a protein–protein binding domain and so we used the yeast two-hybrid system (Fields and Song, 1989) to search for interacting proteins. The full-length ORF of ZNF198 was cloned as the ‘bait’ into the pAS2.1 vector. After confirming that this construct did not autoactivate the reporter genes in the CG1945 yeast host strain, this bait was used to screen a ‘matchmaker’ fetal brain cDNA library (Clontech). Approximately  $10^6$  transformants were screened on HIS<sup>-</sup> selective media containing 10 mM 3-amino triazole. In all, 12 clones were identified which activated  $\beta$ -galactosidase using the colony lift assay and subsequently also passed the mating assay test (see Materials and methods). The human cDNAs in these clones were isolated and sequenced. From this analysis, we identified different cDNAs from the same gene in several different clones which was homologous to the *Saccharomyces cerevisiae* RAD6 ubiquitin-conjugating enzyme. RAD6 was originally identified in yeast as important in postreplication DNA repair activity. The human homologues have been isolated and are called HHR6 of which there are two subtypes, HHR6A and HHR6B (Koken *et al.*, 1991). Owing to the very high homology (>95%) of these two genes, it was not possible to determine unequivocally from the two hybrid studies which of them was involved (see below).

### IP analysis of ZNF198 protein complexes

To confirm these novel interactions of ZNF198 with HHR6, we created GFP-tagged constructs of ZNF198 in the pEGFP-C2 vector, and stably transfected this construct into HEK 293 cells. In all of these experiments, the empty pEGFP-C2 vector was used as a control. Several individual colonies were isolated and Western blot analysis using an anti-GFP antibody identified a 177 kDa product, which is the predicted size of the ZNF198–GFP fusion protein. Localization of the GFP–ZNF198 fusion protein was next analysed using fluorescence microscopy. As shown in Figure 1, the full-length, GFP-tagged ZNF198 sublocalizes to punctate regions within the nucleus. This observation is consistent with that of Ollendorff *et al.* (1999), who used a myc-tagged ZNF198 gene. These results demonstrated that the addition of the GFP tag at the N-terminal end of the ZNF198 gene did not interfere with the correct

localization of the protein. Using an anti-GFP antibody we were then able to IP the ZNF198 protein and resolve the coimmunoprecipitated proteins on an SDS–PAGE. To determine whether the HHR6A and 6B proteins were present in the IPs, the Western blots were then probed with two antibodies kindly provided by Dr Hoeijmakers (Netherlands). One of these antibodies identified the HHR6B protein, while the other detected both HHR6A and 6B. Unfortunately, this combination of antibodies does not specifically identify the HHR6A protein, but it is generally accepted (Xin *et al.*, 2000) that both proteins function as a complex. The results shown in Figure 2 clearly demonstrate that HHR6 can be detected in the IP for ZNF198, thus confirming the yeast two-hybrid analysis and demonstrating the interaction between these proteins.

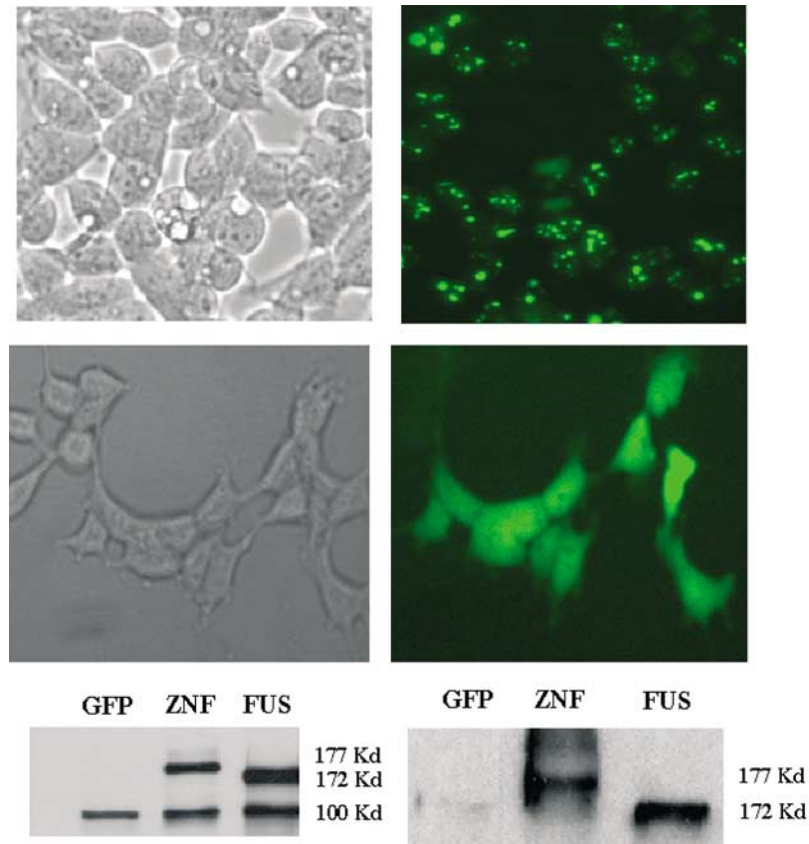
### Stable expression of the ZNF198/FGFR1 fusion kinase

The structural rearrangement seen in MPD fuses the ZNF198 zinc-finger domain with the tyrosine kinase domain of FGFR1. We have recreated this fusion gene in the same GFP vector used for the ZNF198 gene and transfected it into HEK 293 cells. Stable transformants were isolated and expression of the fusion gene was demonstrated by RT–PCR using primers previously described by Still and Cowell (1998). The antipeptide ZNF198 antibody recognized both the full-length ZNF198 gene product as well as the fusion gene product (Figure 1). Analysis using both the anti-GFP and the ZNF198-specific antibody described above, demonstrated production of the appropriately sized proteins (Figure 1). Expression of the fusion kinase gene was also confirmed using the commercially available, C-terminal domain-specific, FGFR1 antibody which is the region maintained in the fusion kinase gene (data not shown) as well as the GFP antibody (Figure 1). Interestingly, the fusion protein shows a more generalized distribution throughout the cell cytoplasm and nucleus with a clear absence of the nuclear punctate staining seen for ZNF198. These results vary with those in previous reports which suggested that the fusion gene was localized exclusively in the cytoplasm (Popovici *et al.*, 1999; Smedley *et al.*, 1999). These other studies, however, involved transient transfection into nonhuman cells. Indeed, in transient transfections we also found that the fusion protein was located in the cytoplasm. However, following selection of stable transformants, expression of ZNF198/FGFR1 is seen throughout the cell.

To determine whether HHR6A and 6B bind to the fusion protein, we again performed IP experiments using the anti-GFP antibody followed by Western blot analysis using the two antibodies described above for HHR6. The results are shown in Figure 2 which demonstrates that, like the full-length ZNF198 gene, the ZNF198/FGFR1 fusion gene also interacts with HHR6.

### Binding of the HRAD18 gene to ZNF198

There is evidence in yeast and in human cells that the RAD18 gene forms a complex with the HHR6A and 6B



**Figure 1** Expression of the GFP-tagged ZNF198 proteins indicates a punctate distribution within the nucleus of 293 cells (top panel), whereas expression of the ZNF198/FGFR1 fusion gene is seen distributed throughout the cell (middle panel). Western blot analysis of stably transfected cells using an anti-GFP antibody (lower left) identifies the 177 kDa protein in cells transfected with ZNF198 (lane 2) and a 172 kDa protein (lane 3) in cells carrying the fusion protein. In all experiments, a series of nonspecific bands are seen with a dominant band at approximately 100 kDa. Using the anti-ZNF198 antibody (lower right), specific products are again seen in cells carrying the normal ZNF198 and fusion genes and not in cells carrying the GFP vector alone

proteins (Xin *et al.*, 2000). To determine, therefore, whether RAD18 is also present in the protein complex with ZNF198, we used a commercially available anti-RAD18 antibody (Imgenex, San Diego, CA, USA) to probe the Western blot generated from IP of both the full-length ZNF198 protein, as well as the ZNF198/FGFR1 fusion kinase. As shown in Figure 3, RAD18 apparently only co-IPs with the full-length protein and not the fusion protein.

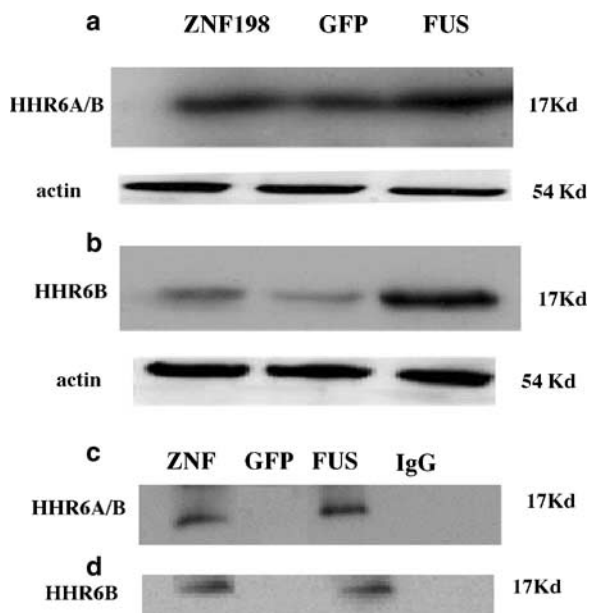
#### *Survival studies of ZNF198-expressing cells to UV damage*

Since the HHR6 and RAD18 genes have been associated with damage repair following UV irradiation, we investigated whether 293 cells overexpressing either the full-length ZNF198 gene (which localizes to the nucleus) or the fusion gene (which stays predominantly in the cytoplasm) affects their ability to repair UV damage. To do this, we measured cell survival after 72 h postirradiation with various doses of UVB. Since we have shown that the zinc-finger motif of ZNF198 has a dimerization function (Baumann *et al.*, 2003), it might have been expected that the fusion protein would act in a dominant-negative manner. The results of these

experiments are shown in Figure 4. Cells transfected with either the empty GFP vector or the full-length ZNF198 show a 50% death rate after a dose of 500 J/m<sup>2</sup>, which is consistent with results using primary human fibroblasts (Tateishi *et al.*, 2000). In contrast, 95% of cells expressing the fusion protein are killed with the same dose.

#### **Discussion**

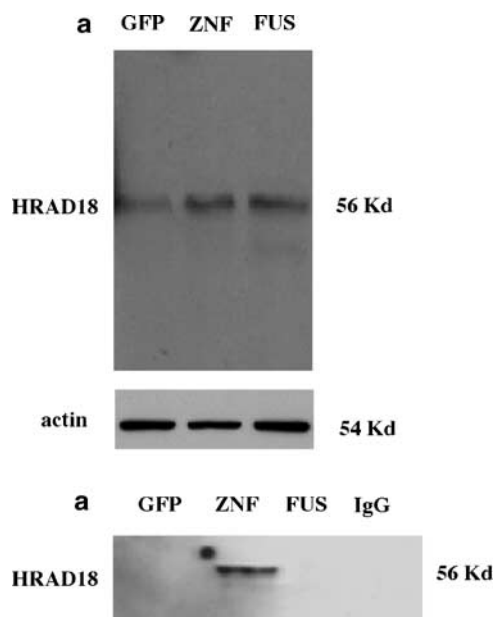
The identification of a novel fusion gene, especially in a leukemia, usually implies that the resultant chimeric protein is promoting the malignant phenotype. However, it is also possible that an altered normal function of one or both members of the fusion partners may also contribute to tumorigenesis. Clearly, a better understanding of the normal function of the fusion partners helps in this determination. We have shown that the ZNF198/FGFR1 fusion kinase is a powerful transforming oncogene and reorganizes the transcription profile of the cells expressing it (Baumann *et al.*, 2003). Our demonstration here that ZNF198 can bind to proteins that are important in the control of DNA repair, cell cycle, chromosome segregation and embryonic



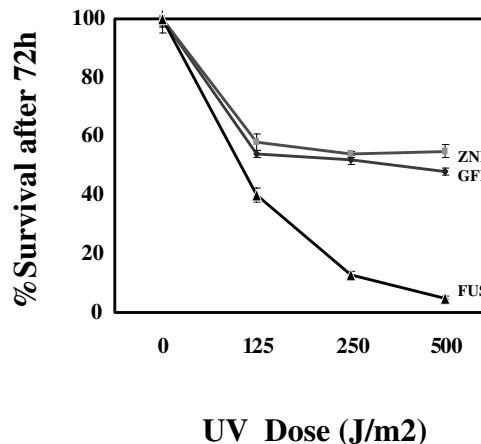
**Figure 2** Analysis of the interaction between HHR6 and the ZNF198 and the fusion ZNF198/FGFR1 (FUS) genes. When Western blots containing whole-cell lysates of 293 cells are probed with either the HHR6A/B antibody (a) or the antibody specific to HHR6B alone (b), cells stably transfected with either the GFP gene alone (GFP), the full-length ZNF198 or the ZNF198/FGFR1 fusion demonstrate endogenous expression of the genes compared with actin (shown below each panel). When anti-GFP antibodies (c and d) are used to immunoprecipitate the transfected proteins, subsequent Western blot analysis demonstrates the presence of the HHR6 proteins using the mixed HHR6A/B (c) as well as the HHR6B (d) antibodies. In this case, the HHR6 proteins are detected in IPs for both the ZNF198 and the fusion (FUS) genes

development provides the first clues to its function and raises the possibility that disturbances in these pathways may also contribute to tumorigenesis.

In *S. cerevisiae*, RAD6 is a ubiquitin-conjugating enzyme shown to have DNA repair activity. Loss of its function increases the sensitivity to many different kinds of DNA damage (Prakash, 1981; Lawrence, 1994). During DNA replication, polymerases cannot bypass unrepaired lesions, resulting in the stalling of the replication complex (Bailly *et al.*, 1997a). It was recently shown that the RAD18 gene is also required for post-DNA replication repair in human cells (Tateishi *et al.*, 2000). RAD6 forms a complex with RAD18, a zinc-finger/ring-finger containing protein which in turn recognizes and binds to single-stranded DNA (Bailly *et al.*, 1997a, b). It has been suggested that RAD18 is responsible in some way for directing RAD6 to sites of DNA damage, which results in the correction of the stalled polymerase complex (Bailly *et al.*, 1997a). Both RAD18 and RAD6 in yeast, and their counterparts in human cells, are localized to the nucleus as expected (Koken *et al.*, 1991; Van der Laan *et al.*, 2000). RAD6 has also been shown to polyubiquitinate histones H2A and H2B at specific lysine residues, which then targets them for degradation and also regulates H3 methylation and gene silencing in yeast (Sung *et al.*, 1988; Koken *et al.*, 1991; Sun and Allis, 2002). Thus, it is possible



**Figure 3** In Western blot analysis of 293 cells transfected with either GFP alone, the full-length GFP-ZNF198 gene or the GFP-fusion (FUS) demonstrates the constitutive expression of the HRAD18 gene (a). Immunoprecipitates using an anti-GFP antibody from the same three stably transfected cell lines, when probed with the anti-HRAD18 antibody, detects this protein only in the cells containing the ZNF198 gene (b)



**Figure 4** Survival analysis following UV irradiation of 293 cells expressing various gene constructs. HEK 293 cells transfected with the GFP vector alone show a nonlinear response to the exposure to UVB irradiation. At a dose of 500 J/m<sup>2</sup>, 50% of cells are killed as determined by Trypan blue exclusion. An identical dose response was seen for cells overexpressing the full-length ZNF198 gene. Cells expressing the fusion kinase gene show a more linear dose response with 95% of cell killed after a dose of 500 J/m<sup>2</sup>

that RAD6 can modify chromatin structure in order to facilitate repair after mutagenesis. RAD6 is highly conserved across species with an N-terminal globular domain and a C-terminal acidic domain which directs ubiquitination. It has recently been demonstrated (Hoegge *et al.*, 2002) that RAD6 mediates ubiquitination of PCNA, which is absolutely required for the cellular DNA damage repair mechanism in yeast. In humans,

HHR6A and HHR6B, which show 95% homology to each other, are the two homologues of yeast RAD6 and are located in Xq24 and 5q23–31, respectively. HHR6A and 6B, alone or in combination, can complement the loss of function of RAD6 in yeast cells following UV damage (Koken *et al.*, 1991). ZNF 198 is also localized to the nucleus which is consistent with a DNA repair function, although its fusion with FGFR1 results in a more diffuse cellular distribution which might alter this function.

The ZNF198 zinc-finger motif acts as a dimerization motif in the fusion protein resulting in activation of the FGFR1 kinase domain (Baumann *et al.*, 2003). In the normal ZNF198, however, the zinc-finger presumably also mediates dimerization and is necessary to facilitate interactions with other proteins. It was possible, therefore, that the fusion protein may act in a dominant-negative way by sequestering the normal protein and so interfere with its ability to bind its partner proteins. Although HHR6A and HHR6B can still bind to the fusion protein, one surprising observation was that the fusion protein did not apparently bind RAD18, whereas ZNF198 did. In our survival studies, it was clear that cells expressing the fusion kinase protein were less efficient in repairing UV damage supporting a dominant-negative role for the fusion kinase related to this function. While it is possible that secondary conformation of the fusion protein excludes RAD18 binding, or that RAD18 binds to a region of ZNF198 not represented in the fusion protein, we feel that the IP observation is more related to the cellular compartmentalization of the respective genes. HHR6 can be found in the cytoplasm where the fusion protein is predominantly localized. In contrast, RAD18 is exclusively nuclear, where it is presumably recruited to the repair complex. Thus, the fusion protein, by interacting with the endogenous ZNF198 protein sequesters HHR6 to the cytoplasm which, in turn, possibly prevents the efficient assembly of the repair complex in the nucleus.

Since RAD6 is absolutely required in the protein complex for the DNA repair function in yeast (Bailly *et al.*, 1994) and human cells (Tateishi *et al.*, 2000), it is

likely that leukemic cells harboring the ZNF198/FGFR1 fusion protein, as for the 293 cells reported here, are less efficient in this capacity. Clearly, without an efficient DNA repair capability, these cells are potentially more susceptible to the development of mutations which can promote more rapid cell growth. One observation from the RAD18 knockout in yeast was that these cells became more prone to aberrant mitosis which could lead to aneuploidy. In the human variant form of MPD, although there are no reported large-scale chromosome abnormalities, trisomy 21 is frequently seen in the AML tumors in these patients.

Recent studies in breast cancer have also implicated the RAD6/RAD18 complex in the development of the malignant phenotype. HHR6B was shown to be over-expressed in metastatic cells from mouse and human breast cancer cells (Shekhar *et al.*, 2002). In transfection studies into nonmalignant mammary epithelial cells, multinucleation, centrosome amplification and anchorage independence resulted from overexpression of HHR6B. This analysis identified RAD6 in the cytoplasm with diffuse or punctate staining in the nucleus in nonmetastatic cells, but large aggregates formed in the nuclei of metastatic breast cancer cells.

The results presented here, therefore, demonstrate that ZNF198 is part of a protein complex which is thought to be involved in DNA repair, and a more diverse role in maintaining genome stability. It is possible, therefore, that the translocation event in MPD not only activates a powerful oncogenic tyrosine kinase (Baumann *et al.*, 2003) but may also have other drastic consequences because of abnormal chromosome segregation. Clearly identifying other proteins that interact with ZNF198 will be important in understanding its role in genomic instability and tumorigenesis.

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