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Review Article

Molecular Mechanism of Activation and Superactivation of Ret Tyrosine Kinases by Ultraviolet Light Irradiation

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ABSTRACT

The catalytic activities of Ret tyrosine kinases as the products of oncogene *RET* with multiple endocrine neoplasia type 2A (Ret-MEN2A) or 2B (Ret-MEN2B) mutations and the hybrid gene from c-*RET* and *RFP* (Rfp-Ret) were higher than those of c-Ret. We demonstrated that ultraviolet light (UV) irradiation induced activation of c-Ret and superactivation of genetically mutated, and thereby constitutively activated, Ret-MEN2A, Ret-MEN2B, and Rfp-Ret. We found that small proportions of c-Ret and Ret-MEN2B and a large proportion of MEN2A were dimerized due to disulfide bonds and that high kinase activity resided in these fractions. The UV-induced activation of c-Ret and superactivation of Ret-MEN2A and Ret-MEN2B were then shown to be closely associated with promotion of the disulfide bond-mediated dimerization of the Ret proteins. Furthermore, we showed that a large proportion of Rfp-Ret was dimerized or polymerized and that almost all kinase activities resided in the highly polymerized but not dimerized fraction. The UV-induced superactivation of Rfp-Ret was also found to be closely associated with promotion of polymerization but not with dimerization of Rfp-Ret. Further experiments revealed that UV induced intracellular dimerization and activation of the extracellular domain-deleted mutant Ret (Ret-PTC-1). Most importantly, the levels of basal kinase activity and dimerization of Ret-TPC-1-C376A, in which cysteine 376 in the tyrosine kinase domain of Ret-TPC-1 was replaced with alanine, were low and were not increased by UV irradiation. These results suggest that the cysteine at this position works as the primary target of dimerization of Ret proteins inside the cell for both the maintenance of the basal kinase activity and its promotion by UV, possibly in co-operation with the cysteine(s) in the extracellular domain of Ret-MEN2A and Rfp-Ret, which is the target of dimerization and polymerization outside the cell. The potential biological significance of the UV-mediated superactivation of mutant Ret through the newly proposed mechanism in oncogenesis is discussed.
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INTRODUCTION

GENETIC FACTORS play a crucial role in tumorigenesis, and tumors are believed to develop by stepwise involvement of multiple oncogenes. A proto-oncogene develops to an oncogene because of a mutation caused by a number of environmental elements, such as X-ray, ultraviolet light (UV), and mutagenic chemicals. Some oncogenes such as *RET* are, however, transmitted from mother to offspring in the germ line. The proto-oncogene c-*RET* encodes a receptor-tyrosine kinase with a cadherin-like motif in the extracellular domain (Takahashi, 1995, 1997). c-Ret is an essential signaling component for renal organogenesis and enteric neu-

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rogenesis (Schuchardt *et al.*, 1994; Takahashi, 1997). Germ line mutations of c-*RET* are associated with the development of multiple endocrine neoplasia type 2A (MEN2A) and type 2B (MEN2B) (Fig. 1A), and rearrangement of this gene is frequently found in human papillary thyroid carcinomas (PTC) (Fig. 1B) (Grieco *et al.*, 1990; Ishizaka *et al.*, 1990; Donis-Keller *et al.*, 1993; Mulligan *et al.*, 1993; Carlson *et al.*, 1994; Hofstra *et al.*, 1994). We have established oncogene *RET* (*RFP-RET*) transgenic mouse lines as an animal model of these hereditary cancers (Iwamoto *et al.*, 1991; Kato *et al.*, 1998b).

Hereditary cancers, however, develop usually after a long latent period. This was also true for the melanocytic tumors that slowly developed with a latent period of 3–4 months after birth in the *RFP-RET* transgenic mice (Kato *et al.*, 1998a,b). These facts suggest that environmental factors play a crucial role in hereditary oncogene-mediated tumorigenesis. Earlier reports have emphasized the role of DNA damage caused by UV or other environmental elements, and few studies have demonstrated the role of conformational changes of the oncogene products by these elements in tumorigenesis. Recent studies have provided evidence that environmental elements represented by UV promote autophosphorylation/activation of receptor-type and non-receptor-type protein tyrosine kinases (PTKs), including epidermal growth factor receptor (EGFR), insulin receptor, c-Src, and ZAP-70 (Devary *et al.*, 1992; Schieven *et al.*, 1994; Warmuth *et al.*, 1994; Coffer *et al.*, 1995). These events may be involved in the whole mechanism of oncogenesis. Little is known, however, about the mechanism of UV or other environmental elements that induce PTK activation, and the primary target of the environmental elements may (Coffer *et al.*,

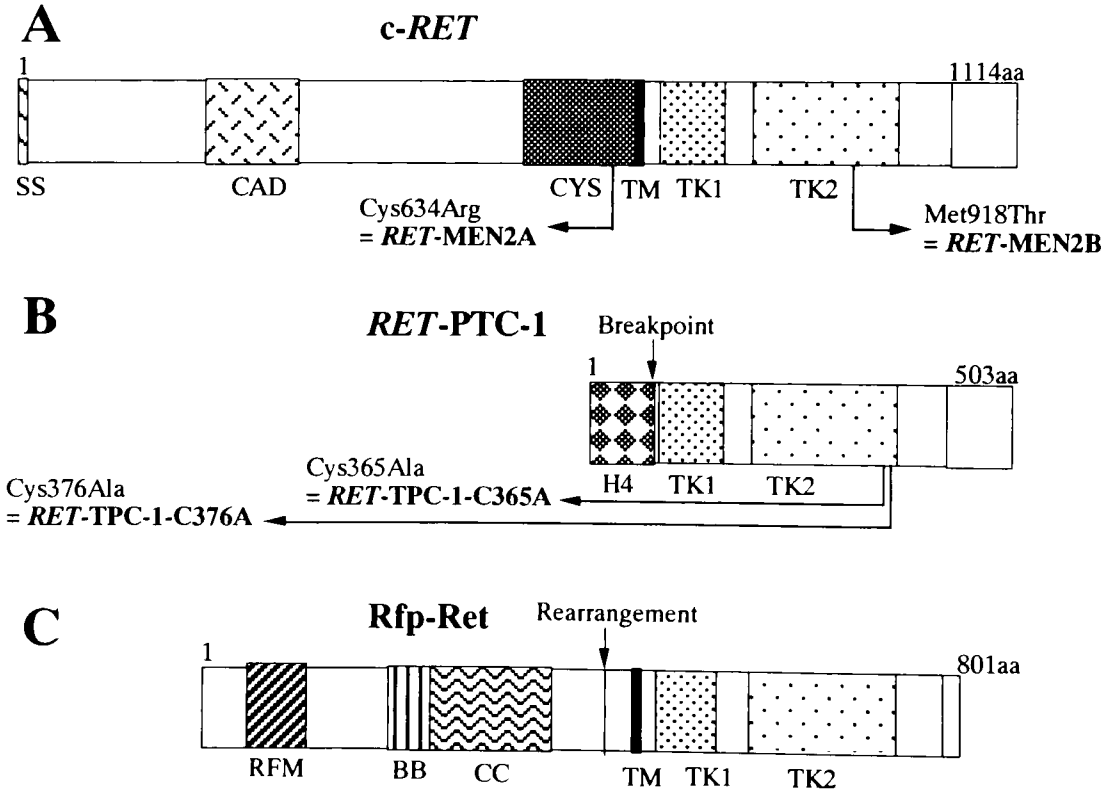

FIG. 1. Schematic illustration of constructs of mutant *RET* cDNAs. (**A**) *RET* cDNA encoding a long isoform (1,114 amino acids) in which cysteine at codon 634 was replaced by arginine (C634R; *RET*-MEN2A) or methionine at codon 918 by threonine (M918T; MEN2B). (**B**) *RET*-PTC-1 in which cysteine at codon 365 or 376 was replaced by alanine (Ret-TPC-1-C365A, Ret-TPC-1-C376A). (**C**) *RFP-RET* cDNA. SS, Signal sequence; CAD, cadherin-like domain; CYS, cysteine-rich region; TM, transmembrane domain; TK1, tyrosine kinase domain 1; TK2, tyrosine kinase domain 2; RFM, RING finger motif; BB, B box; CC, coiled-coil region; aa, amino acids.

1995; Rosette and Karin, 1996) or may not (Knebel *et al.*, 1996) be the kinase protein itself.

Recently, we demonstrated that UV irradiation promotes the disulfide bond-mediated dimerization of Ret proteins in close association with activation of c-Ret and the second-step activation (superactivation) of constitutively activated Ret-MEN2A and Ret-MEN2B kinases (Kato *et al.*, 2000). Rfp-Ret was found to have a unique structural property in that the amino-terminal half of Rfp with RING finger motif (RFM) was fused to the truncated Ret receptor tyrosine kinase (Fig. 1C) (Hasegawa *et al.*, 1996), but the reason why Rfp-Ret is constitutively activated and works for tumor development has not yet been elucidated. In this article, we briefly review our recent study results on the molecular mechanism of activation and superactivation by UV irradiation of RET kinases, including some previously unpublished data on Rfp-Ret.

UV IRRADIATION INDUCES SUPERACTIVATION OF MUTANT RET KINASES

Previously, we examined the effect of UVB irradiation (600 J/m^2) to NIH-3T3 cells that had been transfected with c-*RET*, *RET*-MEN2A, or *RET*-MEN2B on the levels of autophosphorylation and kinase activity of Ret (Kato *et al.*, 2000). The catalytic activities of the constitutively activated Ret-MEN2A and Ret-MEN2B were found to be further up-regulated by UVB. We further examined the effect of UV irradiation on the kinase activity of Rfp-Ret. As shown in Fig. 2, kinase activity of Rfp-Ret (Fig. 2A, lane 2) was already elevated (three to four times) by recombination of coding DNA, as compared with that of c-Ret (in Fig. 2A, lane 1). UVB further up-regulated the kinase activity of Rfp-Ret for both autophosphorylation and MBP phosphorylation (Fig. 2C). These results suggest that UV irradiation induces superactivation of Rfp-Ret kinase as well as Ret-MEN2A and Ret-MEN2B through a mechanism secondary to the genetic one.

UV IRRADIATION PROMOTES DIMERIZATION OR POLYMERIZATION OF RET

Earlier reports suggested that the promotion of the dimerization of Ret by the MEN2A-type point mutation (Asai *et al.*, 1995; Santoro *et al.*, 1995) or a ligand (Chiariello *et al.*, 1998) activates its kinase activity. Recently, we demonstrated that UV promotes dimerization of Ret proteins as a mechanism of activation of c-Ret and superactivation of Ret-MEN2A and Ret-MEN2B by UV (Kato *et al.*, 2000). As shown in Fig. 3, a small amount (3–4%) of c-Ret formed dimers under unreducing condition (Fig. 3A, lane 1), and 40% of the total autophosphorylated Ret protein resided in this dimerized position (Fig. 3B, lane 1). This demonstrated a close relation between dimerization and autophosphorylation/activation of c-Ret at their background levels. UV irradiation promoted dimerization of c-Ret up to 8–9% (two- to three-fold increase; Fig. 3A, lane 2), in close association with an increase in the autophosphorylated Ret (Fig. 3B, lane 2). We also demonstrated that a greater portion (30–40%) of Ret-MEN2A

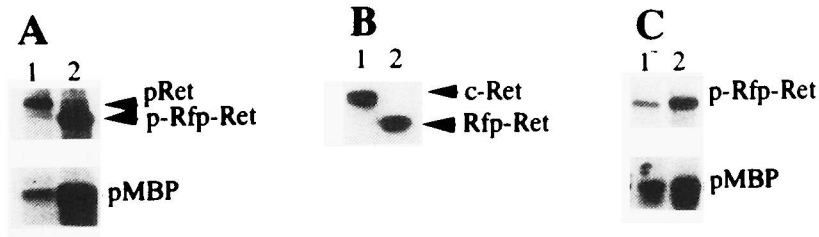

FIG. 2. UV irradiation induces superactivation of Rfp-Ret. Lysates from the NIH-3T3 cells transfected with c-*RET* (lane 1 in **A** and **B**) or *RFP-RET* (lane 2 in **A** and **B, C**) were subjected to Western blotting with anti-Ret antibody (**B**) or to *in vitro* kinase assay (**A,C**) after immunoprecipitation with anti-Ret antibody. (**C**) Lane 1, Sham irradiation; lane 2, 5 min after 600 J/m^2 of UVB irradiation. pRet, Autophosphorylated c-Ret; p-Rfp-Ret, autophosphorylated Rfp-Ret; pMBP, phosphorylated MBP.

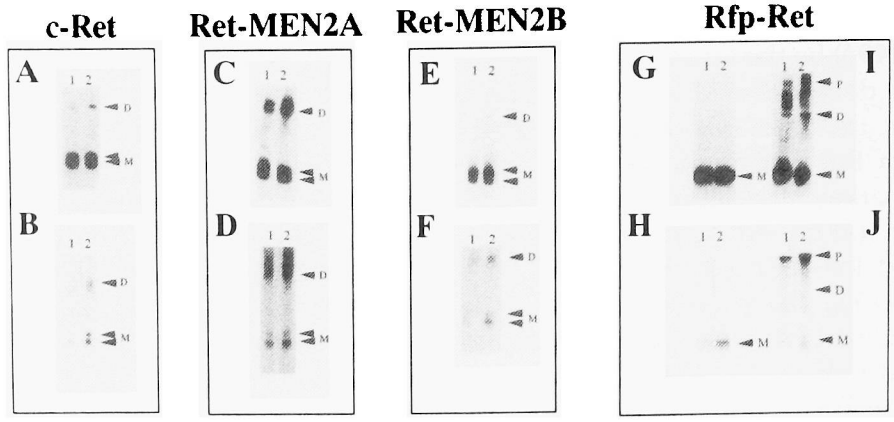

FIG. 3. UV promotes dimerization and polymerization of Ret. Lysates from NIH-3T3 cells transfected with c-*RET* (**A,B**), *RET*-MEN2A (**C,D**), *RET*-MEN2B (**E,F**), or *RFP-RET* (**G–J**) after sham or UV irradiation were subjected to Western blotting with anti-Ret antibody (**A,C,E,G,I**) or to *in vitro* kinase assay (**B,D,F,H,J**) after immunoprecipitation with anti-Ret antibody. SDS-PAGE was done under reducing (**G,H**) or nonreducing (**A–F,I,J**) conditions. (**A–J**) Lane 1, Sham irradiation; lane 2, 5 min after 600 J/m^2 of UVB irradiation. M, monomer Ret; D, dimer Ret; P, highly polymerized Ret. (Reprinted in part from *Molecular Biology of the Cell*, 2000, volume 11, 93–101, with permission by the American Society for Cell Biology.)

formed dimers due to disulfide-bonded cross-linkage of Ret proteins in their extracellular domain with a mutation at cysteine 634, and 60–70% of the total autophosphorylated Ret protein resided at this dimerized position (Fig. 3C,D, lane 1). UV irradiation further promoted dimerization of Ret-MEN2A up to 50–60% (Fig. 3C, lane 2), in close association with an increase in the autophosphorylated Ret (Fig. 3D, lane 2). A strong association of the enzyme activity for the autophosphorylation and dimerization of the kinase proteins, and their co-ordinated promotion by UV irradiation, was also observed with Ret-MEN2B, although only a small amount (1–2%) of Ret-MEN2B formed dimers before UV irradiation (Fig. 3E,F).

We further examined the relation between dimerization and kinase activity for Rfp-Ret. As also shown in Fig. 3, 5–6% of Rfp-Ret was located in the dimerized position and 35–40% of Rfp-Ret was in the polymerized position, including 6–7% of the highly polymerized one under unreducing conditions (Fig. 3I, lane 1). Surprisingly, no demonstrable amount of autophosphorylated Ret resided in the dimerized position (Fig. 3J, lane 1), whereas >90% of the total autophosphorylated Ret resided in the highly polymerized position (Fig. 3J, lane 1). This result suggests that a redox-related high-grade polymerization of Ret proteins due to a yet-undefined mechanism underlies the activation of Rfp-Ret as a hybrid gene product. UV irradiation promoted the dimerization and polymerization of Rfp-Ret up to 8–10% and 45–50%, respectively. Notably, the fraction of high-grade polymerization increased up to 15–20% (three-fold increase; Fig. 3I, lane 2), and the majority of the accelerated autophosphorylation resided in this fraction of Rfp-Ret (Fig. 3J, lane 2). These results suggest that UV induces superactivation of Rfp-Ret kinases through promotion of their high-grade polymerization by a redox mechanism.

DETERMINATION OF THE LOCATION OF CYSTEINE RESIDUE(S) THAT IS RESPONSIBLE FOR POLYMERIZATION AND ITS UV-MEDIATED PROMOTION OF RET

Recently, we tried to determine partially the submolecular target of UV in promoting dimerization and activation of Ret, and we found that UVB irradiation increased autophosphorylation (9–10 times) and substrate phosphorylation (five to six times) levels of Ret-PTC-1 with deletion of the extracellular domain (Kato *et al.*, 2000). These results suggested that nothing in the extracellular domain of Ret kinase serves as the indispensable submolecular target of UV for kinase activation of the kinase. We then

demonstrated that only a small amount (1–2%) of Ret-PTC-1 was dimerized before UV irradiation, but about 80% of the total autophosphorylated Ret resided in this dimerized position, and that UV irradiation promoted dimerization of Ret-PTC-1 up to 5–8% (three- to five-fold increase) in close association with the elevation of the autophosphorylated Ret (Kato *et al.*, 2000). These results suggested that the intracellular domain of Ret may be the primary submolecular target of UV in promotion of dimerization and activation of the Ret kinase.

We next examined the possible target amino acid(s) of UV in Ret-PTC-1 for dimerization and activation. We focused our study on two cysteines in the kinase domain 2 (cysteine 365 and cysteine 376 of Ret-PTC-1), which are conserved among different protein tyrosine kinases (Fig. 4; Veillette *et al.*, 1993), by using prepared *RET*-PTC-1-C365A and *RET*-PTC-1-C376A transfectants (see Fig. 1B). As we recently reported (Kato *et al.*, 2000), although there was not much difference in the background kinase activity between the original Ret-PTC-1 and Ret-PTC-1-C365A, the background Ret kinase activity of Ret-PTC-1-C376A was much lower than that of Ret-PTC-1-C365A or the original Ret-TPC-1. Kinase activities of the original Ret-PTC-1 and Ret-PTC-1-C365A, but not that of Ret-PTC-1-C376A, were up-regulated by UVB irradiation with promotion of dimer formation. These results suggested that Ret-PTC-1 cysteine 376 is the major amino acid as the target of UV for both promotion of dimerization and activation of Ret-PTC-1. Equivalent to Cys376, which was shown to be a crucial amino acid for disulfide-bond-mediated dimerization and its promotion by UV for Ret-PTC-1, may also play a key role in promotion of polymerization and activation of Rfp-Ret by UV, which carries the conserved cysteine in its intracellular domain. A large proportion of Rfp-Ret proteins, however, formed complex polymers rather than simple dimers, and the formation of both dimers and polymers was promoted by UV. This suggests that cysteines of Rfp as the artificial extracellular domain of Rfp-Ret co-operate with the conserved intracellular cysteines for formation and its promotion by UV of active complex polymers of Rfp-Ret.

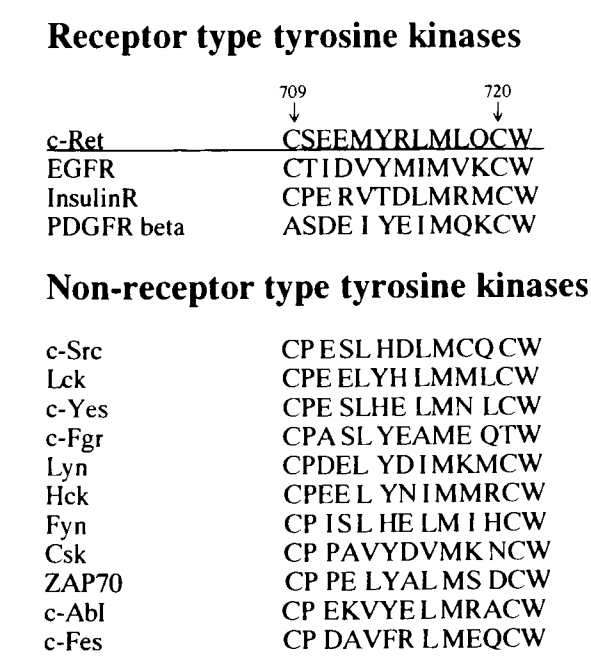

FIG. 4. Cysteines 709 and 720 in c-Ret, which correspond to cysteines 365 and 376 in Ret-PTC-1, are highly conserved throughout the protein tyrosine kinase family. This figure was prepared according to Veillette *et al.* (1993) and Takahashi *et al.* (1987).

FURTHER INSIGHT OF THE MECHANISM OF UV-MEDIATED RET KINASE ACTIVATION

The most intriguing observation in our study was that both c-Ret and mutant Ret more or less form dimers or polymers in close association with the basal kinase activity and that UV irradiation promotes the formation of dimers and polymers, again in association with the elevation of the kinase activity. Background dimer formation and its promotion by UV were also observed with extracellular domain-deleted mutant Ret-PTC-1 proteins.

Although the background levels of dimerization and polymerization of Ret proteins varied among c-Ret, Ret-MEN2A, Ret-MEN2B, Rfp-Ret, and Ret-PTC-1, the extent to which they were promoted by UV was rather constant. This suggests that there may be common UV action sites for different Ret proteins, possibly in their intracellular domains. We have further demonstrated that Cys376, but not Cys365, of Ret-TPC-1 is critical for both basal levels of dimerization and kinase activity of Ret proteins and sensi-

tivity to UV irradiation for their up-regulation (Kato *et al.*, 2000). The equivalent of Cys376 of Ret-TPC-1 is most highly conserved in the sequence of various tyrosine kinases, including EGFR, Src, Lck, Yes, Lyil, Hck, Csk, and Abl (Fig. 4; Takahashi and Cooper, 1987; Veillette *et al.*, 1993), suggesting its critical role in the maintenance and up-regulation of the basal level of catalytic activity of Ret and potentially other tyrosine kinases. Correspondingly, Cys475 of Lck (Veillette *et al.*, 1993) and Cys498 of v-Src (Senga *et al.*, 2000) as equivalents of Cys376 of Ret-TPC-1 have been shown to be crucial for catalytic activity or transforming activity of the kinases. The potential roles of all other cysteines in Ret-PTC-1 in UV-mediated kinase activation are now under examination.

Because of the presence of a high concentration of reduced glutathion (GSH), the intracellular environment is normally maintained in a reducing state. However, a small amount of superoxide is continuously produced during the process of stepwise reduction of O_2 for production of ATP in mitochondria, which produces a small amount of glutathion disulfide (GSSG) and possibly disulfide-bonded intracellular proteins, inducing Ret, as we have demonstrated in this study. On the basis of these observations, we hypothesize that the intracellular redox balance normally regulates the level of disulfide bond formation of intracellular proteins, including PTKs, and that such regulation is normally involved in the mechanism of the initial step of activation of PTKs. Environmental oxidative stress probably mediates this mechanism, as represented by UV-mediated up-regulation of Ret kinase activity. A specific point we have newly introduced in this communication is that polymerization, rather than dimerization, of Rfp-Ret proteins was closely associated with up-regulation of the kinase activity, whereas up-regulation of the kinase activity of c-Ret, Ret-MEN2A, and Ret-MEN2B was closely associated with dimer formation. This phenomenon is probably related to the structural property of Rfp-Ret. The RFM (RING finger motif) of Rfp carries a number of cysteines that are normally located beneath the cell membrane under reducing conditions but are probably translocated at an extracellular site of the cell membrane for potential disulfide-bonded cross-linking of Rfp-Ret proteins, leading to polymer formation. It is possible that the extracellular disulfide bond-mediated polymerization of Rfp-Ret proteins makes intracellular cysteines on two proteins in the vicinity produce an intracellular disulfide bond. UV irradiation would promote additional cross-linkage between two polymers at the intracellular cysteines to form a bigger polymer (Fig. 5). Our proposal of co-operation between extracellular and intracellular cysteines for disulfide-bonded complex cross-linkage of kinase proteins has been supported by the observation of a partial reduction in kinase activity of mutant Rfp-Ret in which one of multiple cysteines on RFM of Rfp-Ret was replaced with phenylalanine (Kato *et al.*, unpublished observation).

BIOLOGICAL SIGNIFICANCE OF UV-MEDIATED RET KINASE SUPERACTIVATION

To correlate the above-described superactivation of Ret kinases with cancer development in animals, we examined the effect of UV irradiation on kinase activity of Rfp-Ret and tumor growth in line 192 of *RFP-RET* transgenic mice (Iwamoto *et al.*, 1991; Kato *et al.*, 1999) in which benign melanocytic tumors spontaneously develop. When UVB (30–45 kJ/m^2 per day) irradiation was applied to the benign tumors over a period of 28 weeks, the tumors were found to be transformed to malignant melanomas in the irradiated mice but not in the nonirradiated control mice (Kato *et al.*, unpublished observation). In these tumors, we found a drastic increase in Ret kinase activity. It is well accepted that UV irradiation damages DNA, inducing mutations in multiple proto-oncogenes and tumor suppressor genes for tumor progression (Ziegler *et al.*, 1994). UV irradiation is also known to down-regulate the host defense mechanism against tumor development (Kripke, 1979). These known mechanisms could play crucial roles in the observed malignant transformation of melanocytic tumors after UV irradiation. Nevertheless, the strong connection between superactivation of Ret kinase and malignant transformation of tumors in the UV-irradiated transgenic mice suggests that the su-

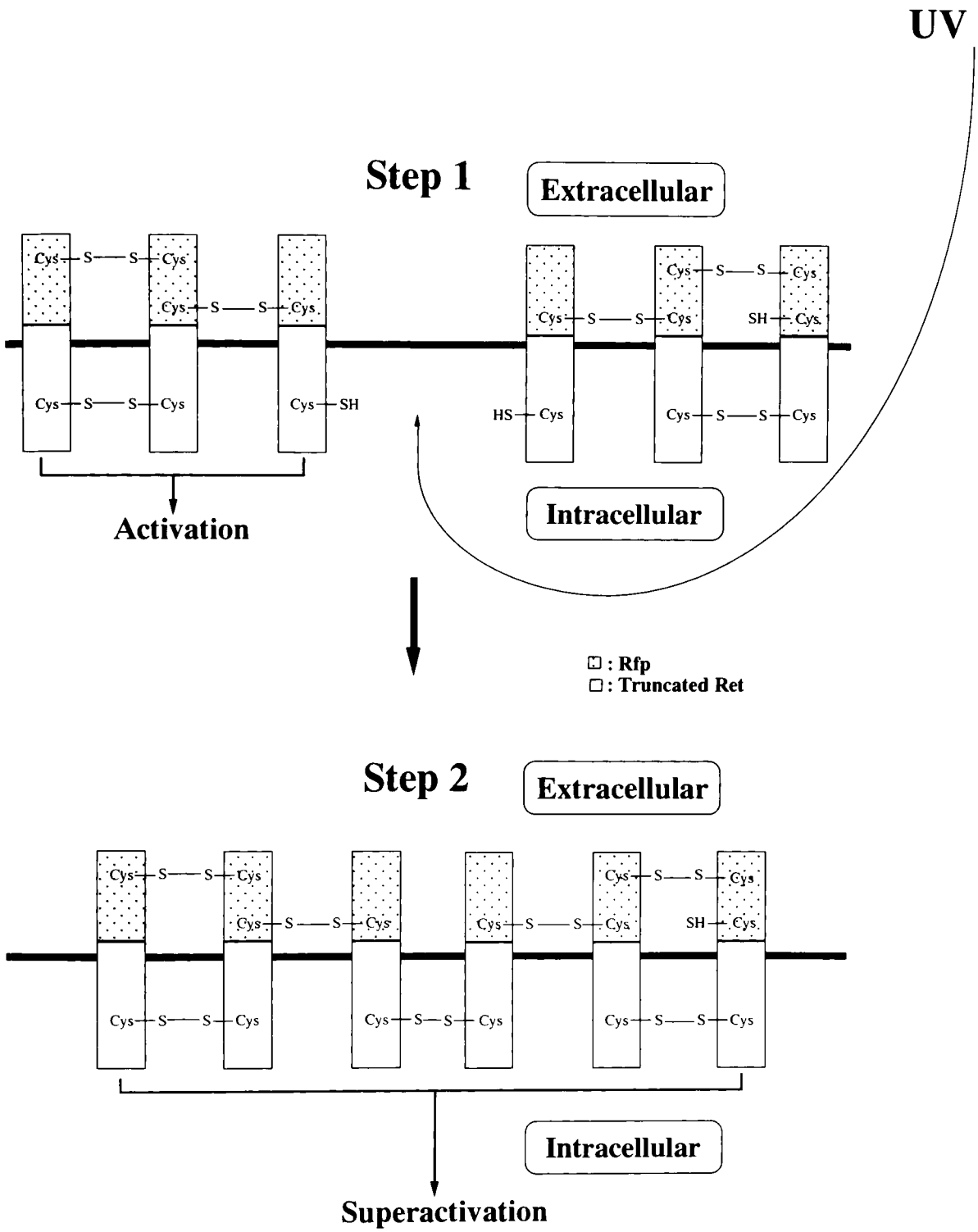

FIG. 5. A hypothetical model for two-step disulfide-bonded cross-linkage of Rfp-Ret proteins at extracellular (step 1) and intracellular (step 2) cysteines for superactivation.

peractivation of a single oncogene product (Ret) by environmental elements such as UV irradiation could also play an important role in stepwise oncogenesis, potentially in concert with previously reported mechanisms.

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ABBREVIATIONS

MBP, Myelin base protein; Ret-MEN2A, *RET* with multiple endocrine neoplasia type 2A mutation; Ret-MEN2B, *RET* with multiple endocrine neoplasia type 2B mutation; UV, ultraviolet light.

REFERENCES

ASAI, N., IWASHITA, T., MATSUYAMA, M., and TAKAHASHI, M. (1995). Mechanism of activation of the *ret* proto-oncogene by multiple endocrine neoplasia 2A mutations. Mol. Cell Biol. **15,** 1613–1619.

CARLSON, K.M., DOU, S., CHI, D., SCAVARDA, N., TOSHIMA, K., JACKSON, C.E., WELLS, S.A. JR., GOODFELLOW, P.J., and DONIS-KELLER, H. (1994). Single missense mutation in the tyrosine-kinase catalytic domain of the *RET* proto-oncogene is associated with multiple endocrine neoplasia type 2B. Proc. Natl. Acad. Sci. USA **91,** 1579–1583.

CHIARIELLO, M., VISCONTI, R., CARLOMAGNO, F., MELILLO, R.M., BUCCI, C., DE FRANCISCIS, V., FOX, G.M., JING, S., COSO, O.A., GUTKIND, J.S., FUSCO, A., and SANTORO, M. (1998). Signaling of the Ret receptor tyrosine kinase through the c-Jun NH2-terminal protein kinases (JNKS): evidence for a divergence of the ERKs and JNKs pathways induced by Ret. Oncogene **16,** 2435–2445.

COFFER, P.J., BURGERING, B.M., PEPPELENBOSCH, M.P., BOS, J.L., and KRUIJER, W. (1995). UV activation of receptor tyrosine kinase activity. Oncogene **11,** 561–569.

DEVARY, Y., GOTTLIEB, R.A., SMEAL, T., and KARIN, M. (1992). The mammalian ultraviolet response is triggered by activation of Src tyrosine kinases. Cell **71,** 1081–1091.

DONIS-KELLER, H., DOU, S., CHI, D., CARLSON, K.M., TOSHIMA, T., LAIMORE, T.C., HOWE, J.R., MOLEY, J.F., GOODFELLOW, P., and WELLS, S.A., JR. (1993). Mutations in the *RET* proto-oncogene are associated with MEN2A and FMTC. Hum. Mol. Genet. **2,** 851–856.

GRIECO, M., SANTORO, M., BERLINGIERI, M.T., MELILLO, R.M., DONGHI, R., BONGARZONE, I., PIEROTTI, M.A., DELLA PORTA, G., FUSCO, A., and VECCHIO, G. (1990). PTC is a novel rearranged form of the *ret* proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. Cell **60,** 557–563.

HASEGAWA, N., IWASHITA, T., ASAI, N., MURAKAMI, H., IWATA, Y., ISOMURA, T., GOTO, H., HAYAKAWA, T., and TAKAHASHI, M. (1996). A ring finger motif regulates transforming activity of the *rfp/ret* fusion gene. Biochem. Biophys. Res. Commun. **225,** 627–631.

HOFSTRA, R.M.W., LANDSVATER, R.M., CECCHERINI, I., STULP, R.P., STELWAGEN, T., LUO, Y., PASINI, B., HOPPENER, J.W.M., VAN AMSTEL, H.K.P., ROMEO, G., LIPS, C.J.M., and BUYS, C.H.C.M. (1994). A mutation in the *RET* proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature **367,** 375–376.

ISHIZAKA, Y., USHIJIMA, T., SUGIMURA, T., and NAGAO, M. (1990). cDNA cloning and characterization of *ret* activated in a human papillary thyroid carcinoma cell line. Biochem. Biophys. Res. Commun. **168,** 402–408.

IWAMOTO, T., TAKAHASHI, M., ITO, M., HAMATANI, K., OHBAYASHI, M., WAJJWALKU, W., ISOBE, K., and NAKASHIMA, I. (1991). Aberrant melanogenesis and melanocytic tumour development in transgenic mice that carry a metallothionein/*ret* fusion gene. EMBO J. **10,** 3167–3175.

KATO, M., LIU, W., YI, H., ASAI, N., HAYAKAWA, A., KOZAKI, K., TAKAHASHI, M., and NAKASHIMA, I. (1998a). The herbal medicine Sho-saiko-to inhibits growth and metastasis of malignant melanoma primarily developed in *ret*-transgenic mice. J. Invest. Dermatol. **111,** 640–644.

KATO, M., TAKAHASHI, M., AKHAND, A.A., LIU, W., DAI, Y., SHIMIZU, S., IWAMOTO, T., SUZUKI, H., and NAKASHIMA, I. (1998b). Transgenic mouse model for skin malignant melanoma. Oncogene **17,** 1885–1888.

KATO, M., LIU, W., AKHAND, A.A., DAI, Y., OHBAYASHI, M., SUZUKI, T., SUZUKI, H., ISOBE, K., TAKAHASHI, M., and NAKASHIMA, I. (1999). Linkage between melanocytic tumor development and early burst of Ret protein expression for tolerance induction in metallothionein-I/*ret* transgenic mouse lines. Oncogene **18,** 837–842.

KATO, M., IWASHITA, T., TAKEDA, K., AKHAND, A.A., LIU, W., YOSHIHARA, M., ASAI, N., SUZUKI, H., TAKAHASHI, M., and NAKASHIMA, I. (2000). Ultraviolet light induces redox reaction-mediated dimerization and superactivation of oncogenic Ret tyrosine kinases. Mol. Biol. Cell **11,** 93–101.

KNEBEL, A., RAHMSDORF, H.J., ULLRICH, A., and HERRLICH, P. (1996). Dephosphorylation of receptor tyrosine kinase as target of regulation by rediation, oxidants or alkylating agents. EMBO J. **15,** 5314–5325.

KRIPKE, M.L. (1979). Speculations on the role of ultraviolet radiation in the development of malignant melanoma. J. Natl. Cancer Inst. **63,** 541–548.

MULLIGAN, L.M., KWOK, J.B., HEALEY, C.S., ELSDON, M.J., ENG, C., GARDNER, E., LOVE, D.R., MOLE, S.E., MOORE, J.K., PAPI, L., PONDER, M.A., TELENIUS, H., TUNNACLIFFE, A., and PONDER, B.A.J. (1993). Germline mutations of the *RET* proto-oncogene in multiple endocrine neoplasia type 2A. Nature **363,** 458–460.

ROSETTE, C., and KARIN, M. (1996). Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science **274,** 1194–1197.

SANTORO, M., CARLOMAGNO, F., ROMANO, A., BOTTARO, D.P., DATHAN, N.A., GRIECO, M., FUSCO, A., VECCHIO, G., MATOSKOVA, B., KRAUS, M.H., *et al.* (1995). Activation of *RET* as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science **267,** 381–383.

SCHUCHARDT, A., D'AGATI, V., LARSSON-BLOMBERG, L., COSTANTINI, F., and PACHNIS, V. (1994). Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor *Ret*. Nature **367,** 380–383.

SCHIEVEN, G.L., MITTLER, R.S., NADLER, S.G., KIRIHARA, J.M., BOLEN, J.B., KANNER, S.B., and LEDBETTER, J.A. (1994). ZAP-70 tyrosine kinase, CD45, and T cell receptor involvement in UV- and H_2O_2-induced T cell signal transduction. J. Biol. Chem. **269,** 20718–2726.

SENGA, T., MIYAZAKI, K., MACHIDA, K., IWATA, H., MATSUDA, S., NAKASHIMA, I., and HAMAGUCHI, M. (2000). Clustered cysteine residues in the kinase domain of v-Src: critical role for protein stability, cell transformation and sensitivity to herbimycin A. Oncogene **19,** 293–279.

TAKAHASHI, M. (1995). Oncogenic activation of the *ret* protooncogene in thyroid cancer. Crit. Rev. Oncogenesis **6,** 35–46.

TAKAHASHI, M. (1997). The role of the *ret* proto-oncogene in human disease. Nagoya J. Med. Sci. **60,** 23–30.

TAKAHASHI, M., and COOPER, G.M. (1987). *ret* transforming gene encodes a fusion protein homologous to tyrosine kinases. Mol. Cell. Biol. **7,** 1378–1385.

VEILLETTE, A., DUMONT, S., and FOURNEL, M. (1993). Conserved cysteine residues are critical for the enzymatic function of the lymphocyte-specific tyrosine protein kinase p56lck. J. Biol. Chem. **268,** 17547–17553.

WARMUTH, I., HARTH, Y., MATSUI, M.S., WANG, N., and DELEO, V.A. (1994). Ultraviolet radiation induces phosphorylation of the epidermal growth factor receptor. Cancer Res. **54,** 374–376.

ZIEGLER, A., JONASON, A.S., LEFFELL, D.J., SIMON, J.A., SHARMA, H.W., KIMMELMAN, J., REMINGTON, L., JACKS, T., and BRASH, D.E. (1994). Sunburn and p53 in the onset of skin cancer. Nature **372,** 773–776.

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3. Shigetsugu Hatakeyama. 2011. TRIM proteins and cancer. *Nature Reviews Cancer* . [CrossRef]
4. Kai Chen , Siobhan E. Craige , John F. Keaney , Jr. . 2009. Downstream Targets and Intracellular Compartmentalization in Nox Signaling. *Antioxidants & Redox Signaling* **11**:10, 2467-2480. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. Ichiro Yajima, Mayuko Kumasaka, Nguyen Dinh Thang, Takeshi Yanagishita, Nobutaka Ohgami, David Kallenberg, Yuji Naito, Toshikazu Yoshikawa, Naomi Sakashita, Masashi Kato. 2009. Zinc finger protein 28 as a novel melanoma-related molecule. *Journal of Dermatological Science* **55**:1, 68-70. [CrossRef]
6. Masashi Kato, Nobutaka Ohgami, Yoshiyuki Kawamoto, Toyonori Tsuzuki, Khaled Hossain, Takeshi Yanagishita, Yuichiro Ohshima, Hideo Tsuboi, Osamu Yamanoshita, Yoshinari Matsumoto, Masahide Takahashi, Izumi Nakashima. 2007. Protective Effect of Hyperpigmented Skin on UV-Mediated Cutaneous Cancer Development. *Journal of Investigative Dermatology* **127**:5, 1244-1249. [CrossRef]
7. Masashi KATO, Kozue TAKEDA, Yoshiyuki KAWAMOTO, Khaled HOSSAIN, Nobutaka OHGAMI, Takeshi YANAGISHITA, Yuichiro OHSHIMA, Yoko KATO, Kyoko OHGAMI, Tatsuya YAMAMORI, Keisuke TATEYAMA, Osamu YAMANOSHITA. 2007. Ultraviole Irradiation-Mediated Malignant Melanoma Induction with RET Tyrosine Kinase Activation. *Nippon Eiseigaku Zasshi (Japanese Journal of Hygiene)* **62**:1, 3-8. [CrossRef]
8. Izumi Nakashima, Kozue Takeda, Yoshiyuki Kawamoto, Yusuke Okuno, Masashi Kato, Haruhiko Suzuki. 2005. Redox control of catalytic activities of membrane-associated protein tyrosine kinases. *Archives of Biochemistry and Biophysics* **434**:1, 3-10. [CrossRef]
9. Izumi Nakashima , Masashi Kato , Anwarul A. Akhand , Haruhiko Suzuki , Kozue Takeda , Kahled Hossain , Yoshiyuki Kawamoto . 2002. Redox-Linked Signal Transduction Pathways for Protein Tyrosine Kinase Activation. *Antioxidants & Redox Signaling* **4**:3, 517-531. [Abstract] [Full Text PDF] [Full Text PDF with Links]