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Review

A mutation in protein phosphatase type 2A as a cause of melanoma progression

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Summary. The BL6 subline was derived from the F10 line, which was derived from the B16 mouse melanoma cell line. BL6 cells are more invasive than F10 cells and differ genetically from F10 cells by an alteration of the gene encoding the B56γ regulatory subunit of protein phosphatase 2A (PP2A). This alteration results in the transcription of mRNA encoding a truncated variant of the B56γ1 isoform (Δγ1). Δγ1 is capable of targeting PP2A to the specific subcellular sites but incapable of promoting the dephosphorylation of specific substrates that is normally mediated by the B56γ subunit-containing PP2A holoenzyme. It thus appears that activities of this type of holoenzymes decrease in cells expressing Δγ1. Recently, we found two possible ways how Δγ1 contributes to the enhanced metastatic potential of BL6 cells. The two ways seemed far away from each other: Δγ1 influenced both the nuclear and cytoplasmic functions of the cell. In the cytoplasm, Δγ1 localized at the Golgi complex and accelerated Golgi-mediated vesicle transport. On the other hand, Δγ1 disturbed the cell-cycle regulation. In response to g-irradiation, protein levels of Δγ1 were markedly increased in BL6 cells. Subsequently the integrity of cell-cycle checkpoint became more aberrant in BL6 cells than that in F10 cells. These two actions of Δγ1 could results in the enhancement of the malignant phenotypes of melanoma cells, as discussed in this review.

Key words: B16 melanoma, PP2A, Genetic instability, Checkpoint, Golgi complex, Migration

Introduction

B16 mouse melanoma cells were originally established from a de novo melanoma tumor (Fidler, 1975). These cells underwent ten rounds of in vivo selection to yield the F10 subline, which in turn went through six rounds of in vitro selection to yield the BL6 subline (Hart, 1979; Poste et al., 1980). Every additional round of selection increased the metastatic potential of the cells. Consequently, while both cells metastasize to the lungs after being injected intravenously into mice, BL6 cells can metastasize to the lungs even after being injected subcutaneously (Hart, 1979; Poste et al., 1980). In an attempt to identify the differences responsible for the heightened metastatic potential of BL6 (Nakaji et al., 1999; Ito et al., 2000a,b; Kataoka et al., 2000; Nakamoto et al., 2001; Watabe et al., 2001), we have been analyzing the gene expression of the two sublines by using an improved method for subtraction. Interestingly, we found that in BL6 cells, a retrotransposon had been inserted into an intronic region of one allele of the gene encoding the B56γ regulatory subunit of protein phosphatase type 2A (PP2A) (Fig. 1) (Ito et al., 2000a).

PP2A consists of a series of serine/threonine phosphatase holoenzymes that are composed of a common dimeric core of invariable catalytic (C) and structural (A) subunits associated with a variable regulatory (B) subunit (Fig. 2) (Usui et al., 1988). The regulatory subunit is extremely diverse because it is constituted by members from at least three unrelated families, namely, PR55 (or simply B), B56 (B'), and PR72 (B'') (Virshup, 2000). Each of these families in turn consists of several subfamilies, each of which contains several proteins resembling each other structurally. B56 appears to be the most complex of the three families as its members are encoded by five distinct mammalian genes that produce at least 13 splicing isoforms. One of these genes is B56γ, which is spliced in three different ways to produce the three isoforms belonging to the B56γ subfamily, namely, B56γ1, γ2 and γ3. In BL6 cells, the 5' part of the original B56γ gene is replaced with the retrotransposon sequence, which results in the abundant expression of a chimeric mRNA species that encodes a mutant protein, termed Δγ1, that lacks the N-terminal 65 amino acid residues of the B56γ1 isoform (Ito et al., 2000a).

It is believed that the regulatory subunits of PP2A control PP2A functions by directing particular trimeric

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PP2A holoenzymes into specific subcellular compartments as well as by enhancing the PP2A phosphatase activity on specific substrates (Kamibayashi et al., 1991, 1994; Ito et al., 2000a; Virshup, 2000). Thus, the diversity of the regulatory subunit of PP2A results in different PP2A holoenzymes with distinct substrate specificities and subcellular localizations (Virshup, 2000). This diversity allows PP2A to regulate a variety of cellular processes. Studies with yeast (Kinoshita et al., 1990), Xenopus oocytes (Millward et al., 1999), and mammalian cells (Yamashita et al., 1990; Kawabe et al., 1997; Okamoto et al., 2002) reveal that one of the most important functions of PP2A is to participate in cell-cycle regulation. Other studies using chemicals that inhibit PP2A, such as okadaic acid, have demonstrated a possible involvement of PP2A in Golgi functions.

Our previous work showed that both B56γ1 and Δγ1 localize in the perinuclear region when they are expressed exogenously as epitope-tagged proteins (Ito et al., 2000a). This work also showed that Δγ1 is incapable of promoting the dephosphorylation of specific substrates that is normally mediated by the B56γ subunit-containing PP2A holoenzyme (Ito et al., 2000a). Thus, while Δγ1 seems capable of acting as a targeting subunit, it lacks the ability to enhance PP2A activity in a substrate-specific manner. In this review, we propose possible two ways how Δγ1 could contribute to the highly malignant phenotypes acquired by BL6 cells.

Δγ1 degrades cell-cycle checkpoint integrity

Two well-known properties of tumor cells are that they are genetically unstable (Lengauer et al., 1998; Cahill et al., 1999) and tend to progress toward increasing malignancy (Chambers and Hill, 1992). Nowell (1976) drew a link between these two characteristics when he predicted that the genetic instability of tumor cells could contribute to malignant progression by generating subclones with different properties. Consistent with this hypothesis, human tumor cells often have mutations in the genes that play essential roles in the cell-cycle checkpoint, an intracellular event that protects the integrity of the genome against genotoxic stress (Sherr, 1996; Lengauer et al., 1998). For example, the p53 tumor suppressor gene, which plays a pivotal role in the checkpoint and is thus called "the guardian of the genome", is mutated in more than half of a wide variety of sporadically occurring human tumors (Sherr, 1996; Lengauer et al., 1998). An intact cell-cycle checkpoint responds to DNA damage by either arresting cell-cycle progression, thereby facilitating DNA repair, or by initiating an apoptotic pathway that eliminates the damaged cell (Paulovich et al., 1997). However, when the checkpoint fails, the cell-cycle progresses despite the DNA damage and can occasionally result in chromosomal aberration (Paulovich et al., 1997) and extensive polyploidy (Illidge et al., 2000). Consequently, checkpoint failure is postulated to contribute to malignant progression by generating multiple subclones in a tumor (Lengauer et al., 1998; Cahill et al., 1999).

Metastasis is the most malignant characteristic of a tumor and can be considered as the final stage of tumor progression (Chambers and Hill, 1992). Studies with experimental tumors that can be transplanted into animals may be useful in examining whether genetic instability contributes to the acquisition of metastatic potentials. It is often possible to select sublines with increased metastatic ability from a parental tumor population (Chambers and Hill, 1992), whose heterogeneity appears to arise from its genetic instability (Shapiro et al., 1981; Chambers and Hill, 1992). One of the best known examples of this is the selection of the

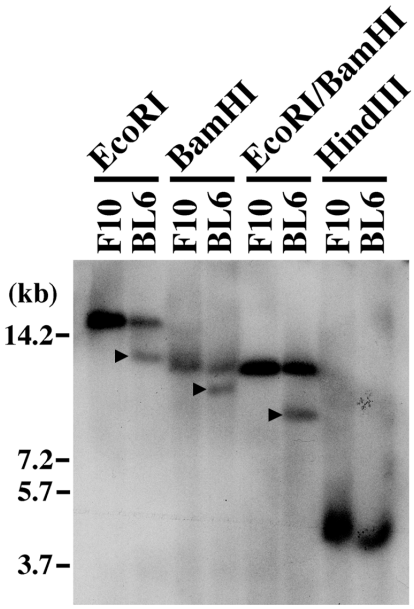

Fig. 1. Southern blot analysis of the B56γ subunit gene in F10 and BL6 cells. Genomic DNA was extracted from F10 and BL6 cells, digested with the restriction enzymes indicated, electrophoresed in agarose gel, and blotted to a nylon membrane. The membrane was hybridized with a B56γ genomic fragment that had been labeled with 32P-dCTP. As shown by arrowheads, BL6 cells have one rearranged locus of the B56γ gene, into which a retrotransposon was inserted.

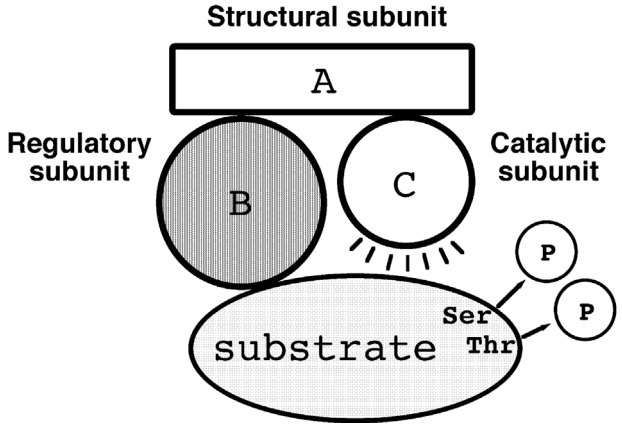

Fig. 2. Schematic presentation of the structure of PP2A. PP2A holoenzymes are structurally a trimer of the three, structural (A), regulatory (B) and catalytic (C), subunits. The B subunit consists of a variety of the isoforms and confers on PP2A holoenzymes distinct substrate-specificities.

F10 and BL6 sublines from B16 mouse melanoma cells (Hart, 1979; Poste et al., 1980). According to the hypothesis proposed by Nowell (1976), it may be that BL6 cells are genetically more unstable than F10 cells and that this has led to the accumulation of a number of genomic alterations that increased their metastatic ability. We hypothesized that Δγ1 may have helped BL6 cells acquire its increased metastatic ability by inducing an aberrant cell-cycle checkpoint and thereby promoting its acquisition of genomic alterations that aid metastasis (Ito et al., 2003a).

Firstly, we attempted to know the direct effect of Δγ1 on the irradiation-induced cell-cycle checkpoint by using NIH3T3 cells, because they respond normally to γ-irradiation. That is, when NIH3T3 cells were grown on culture dishes and then γ-irradiated, they arrested at G1 and G2/M phases for more than a day and returned to their original state within two days (Fig. 3A). In contrast, when g-radiation was delivered over an NIH3T3 clone that stably expressed exogenous Δγ1 proteins (NIH3T3$^{\Delta\gamma1}$), the cells were defective in G2/M cell-cycle arrest and returned to the original state as early as 24 hours (Fig. 3A). Δγ1 expression thus appears to shorten the duration that NIH3T3 cells should be arrested in the G2/M phase in response to g-irradiation.

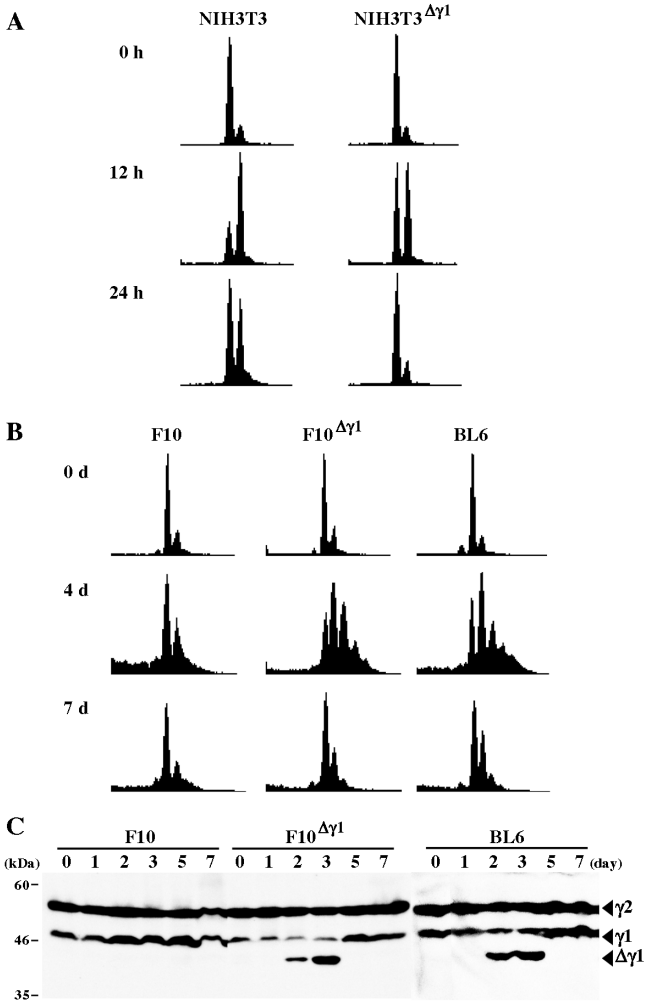

Fig. 3. Cell-cycle responses of Δγ1-expressing cells to g-irradiation. **A.** NIH3T3 and NIH3T3$^{\Delta\gamma1}$ cells were grown in vitro and γ-irradiated by a single 8-Gy dose. At the time periods indicated after irradiation, cells were harvested, stained with propidium iodide, and analyzed by FACScan. **B and C.** F10, F10$^{\Delta\gamma1}$ and BL6 cells were grown in vivo and γ-irradiated by a single 12-Gy dose. On the day indicated after irradiation, tumor cells were removed from the mice and analyzed by FACScan **(B)** and Western blot analyses using an anti-B56γ antibody **(C)**.

Secondly, we established an F10 clone (F10$^{\Delta\gamma1}$) that had been transformed with a plasmid vector expressing Δγ1, and compared cell-cycle responses to γ-irradiation in F10, F10$^{\Delta\gamma1}$ and BL6 cells. Cells were γ-irradiated under two growth conditions, where the cells were grown on culture dishes and in the mouse footpads subcutaneously. Because expression of the Δγ1 protein is detectable in F10$^{\Delta\gamma1}$ and BL6 cells under the latter, but not the former, condition (Ito et al., 2003a), F10$^{\Delta\gamma1}$ and BL6 cells might differ from F10 cells only when the cells were g-irradiated in vivo. In fact, when F10, F10$^{\Delta\gamma1}$ and BL6 cells were grown in vitro and then g-irradiated, all three types of cells displayed an equivalent defect in their checkpoints: the cells exhibited a transient arrest at the G2/M phase and a subsequent accumulation of a polyploid cell fraction. This type of defect is known as the "uncoupling" of the S and M phases (Waldman et al., 1996; Li and Dang, 1999). In contrast, when cells are γ-irradiated *in vivo*, the checkpoint in F10$^{\Delta\gamma1}$ and BL6 cells became more aberrant than that of F10 cells: F10$^{\Delta\gamma1}$ and BL6 cells displayed a high degree of polyploidy that is evident for a longer period, while the cell cycle profile of F10 cells was the same as that after in vitro γ-irradiation (Fig. 3B). Furthermore, the extension of the polyploid period in irradiated in vivo-grown BL6 cells peaked on the fourth day after irradiation and one or two days prior to this, Δγ1 protein levels became markedly increased in F10$^{\Delta\gamma1}$ and BL6 cells (Fig. 3C). Therefore, the emergence of the aberrant cell-cycle checkpoint in irradiated in vivo-grown F10$^{\Delta\gamma1}$ and BL6 cells correlated with the synthesis of the Δγ1 protein. These observations suggest that compared to F10 cells, the checkpoint in F10$^{\Delta\gamma1}$ and BL6 cells is aberrant when the cells were γ-irradiated in vivo, and that this aberration may largely be attributed to the presence of Δγ1.

The cell-cycle response of irradiated in vivo-grown BL6 and F10$^{\Delta\gamma1}$ cells was a somewhat different pattern to that observed in NIH3T3$^{\Delta\gamma1}$ cells. This difference can be explained by the observation that F10 and BL6 cells already suffer from S/M uncoupling, regardless of whether or not Δγ1 is expressed in these cells. Once F10$^{\Delta\gamma1}$ and BL6 cells are grown in vivo and irradiated, they express Δγ1, which inflicts an additional checkpoint fault that causes the cells to undergo multiple rounds of DNA synthesis without arresting long enough in the G2/M phase. The extensive polyploidy exhibited by BL6

and F10Δγ1 cells may thus be attributable to a combination of S/M uncoupling and defective G2/M arrest.

We next examined whether the expression of Δγ1 in *in vivo*-irradiated $F10^{\Delta\gamma1}$ cells could also be associated with their increased metastatic ability. F10, F10Δγ1 and mock-transfected F10 ($F10^{Vec}$) cells were inoculated subcutaneously into the mouse footpads and were g-irradiated with a single subcurative dose (12 Gy) 7 days after inoculation. Subsequently, mice were maintained for 2 months according to the protocol of spontaneous metastasis assays and then autopsied. *In vivo*-irradiated $F10^{\Delta\gamma1}$ cells produced significantly larger numbers of metastatic colonies in the lungs (4.9 colonies/mouse in average) than in vivo-irradiated F10 (0.93 colonies/mouse) and $F10^{Vec}$ (1.2 colonies/mouse) cells and non-irradiated $F10^{\Delta\gamma1}$ cells (1.3 colonies/mouse). Moreover, Δγ1 expression was detectable in the metastatic tumors derived from irradiated $F10^{\Delta\gamma1}$ cells, but not from irradiated F10 cells. These observations support the notion that the expression of Δγ1 correlates with the acquisition of greater metastatic ability.

In contrast to $F10^{\Delta\gamma1}$ cells, BL6 cells did not become more metastatic after in vivo γ-irradiation despite the fact that in vivo γ-irradiation increased Δγ1 protein levels in BL6 cells to the same degree as observed in $F10^{\Delta\gamma1}$ cells. One possible explanation for this is that BL6 cells may have already fully acquired genomic alterations that were generated by Δγ1-induced defect in the cell-cycle checkpoint. It is possible that during the six rounds of in vitro selection of F10 cells that generated the BL6 line, an early event was the retrotransposon insertion into the B56γ gene that allows Δγ1 expression. This rearrangement then allowed the cells to efficiently accumulate multiple mutations in their genomes, some of which promoted metastatic ability. Supporting this is that BL6 cells are still more metastatic than in vivo-irradiated $F10^{\Delta\gamma1}$ cells, whether irradiated or not, as they form >20 metastatic colonies in the lungs when injected subcutaneously into the footpads (Ito et al., 2000a,b). Probably, BL6 cells may have acquired the full range of changes permitted by the Δγ1-induced defect in cell-cycle checkpoint.

Taken together, expression of Δγ1 in response to γ-irradiation appears to be followed by the two events, the degradation of checkpoint integrity and the development of greater metastatic ability. Although the direct causation between the two events remain to be proved, Δγ1 expression may cause the two events by disrupting proper PP2A functioning in the cell-cycle checkpoint. This notion is consistent with the fact that okadaic acid, an inhibitor of protein phosphatases including PP2A, promotes genetic instability and tumorigenicity (Nakagama et al., 1997). Our observations suggest that the B56γ-containing PP2A holoenzymes may be important in guarding genome integrity and that impairment of the function of these holoenzymes may result in malignant progression. Our observations also support the more general hypothesis that genetic instability could promote tumor progression from the non-metastatic to the metastatic state.

Δγ1 accelerates Golgi-mediated vesicle transport

Another action of Δγ1 was suggested by its unique cytoplasmic localization (Ito et al., 2003b). When we stained F10 and BL6 cells with an anti-B56g antibody, we detected specific signals in a limited perinuclear area and wondered if the B56γ subunit might localize at the Golgi complex. This speculation was supported by a previous report by van Lookeren Campagne et al. (1999) who stained neurons with an antiserum recognizing all three isoforms of the B56γ subunit and detected the specific signals in the perinuclear region as well as in the nucleus.

Localization of PP2A holoenzymes at the Golgi complex has long been assumed by the fact that okadaic acid influences the Golgi function. Okadaic acid is a complex fatty acid polyketal (Takai et al., 1987; Bialojan et al., 1988) and inhibits several types of protein phosphatases, including PP1, PP2A, PP4 and PP5 (Takai et al., 1987; Bialojan et al., 1988; Huang et al., 1997; Dobson et al., 2001). When an intact cell is treated with 0.5 mM okadaic acid, the Golgi complex fragments into numerous clusters of Golgi-derived vesicles and tubules that then disperse in the cytoplasm (Thyberg and Moskalewski, 1992; Lucocq et al., 1995; Dinter and Berger, 1998). During the Golgi fragmentation, intracellular vesicle transport arrests (Chou and Omary, 1994; Dinter and Berger, 1998). Since PP1 and PP2A are the major components of the serine/threonine phosphatase activity in the mammalian cell (Cohen, 1991), and since okadaic acid completely inhibits PP2A and PP1 at 1 nM and 5 mM, respectively (Cohen, 1991), it is possible that the Golgi fragmentation caused by the okadaic acid treatment is due to the okadaic acid-induced inhibition of PP2A. In other words, PP2A may be involved in maintaining the morphology of the Golgi complex and in regulating Golgi complex-mediated vesicle transport.

We began by further investigating cytoplasmic localization of the B56γ subunit. Immunocytochemistry revealed that signals specific for this subunit co-localized well with the signals specific for cis-Golgi marker proteins p115 and GM130 (Fig. 4A). This localization was next confirmed by biochemical methods. When nucleus-depleted homogenates of F10 cells were separated in a discontinuous sucrose gradient, a considerable proportion of the B56γ1 and γ2 isoforms was recovered in the fraction enriched with Golgi membranes and trans-Golgi networks while the B56γ3 isoform was not contained in this fraction. Since this Golgi-enriched fraction also contained a considerable amount of the PP2A C subunit, functional PP2A heterotrimers containing the B56γ1 and γ2 isoforms seemed to be present in the Golgi complex.

We examined subcellular localization of Δγ1 by transiently expressing it as a protein tagged with FLAG

(Δγ1-FLAG). Δγ1-FLAG did not colocalize with the cis-Golgi marker p115 but did colocalize well with the trans-Golgi marker TGN38, although its signals extended beyond the region labeled with anti-TGN38 antibody (Fig. 4B). When B56γ1 was transiently expressed as a FLAG-tagged protein, the protein was also detected mainly in the TGN area rather than in the cis-Golgi area. Although the precise localization of Δγ1 and B56γ1 remains to be clarified, both proteins appear to localize to the same compartment of the Golgi complex.

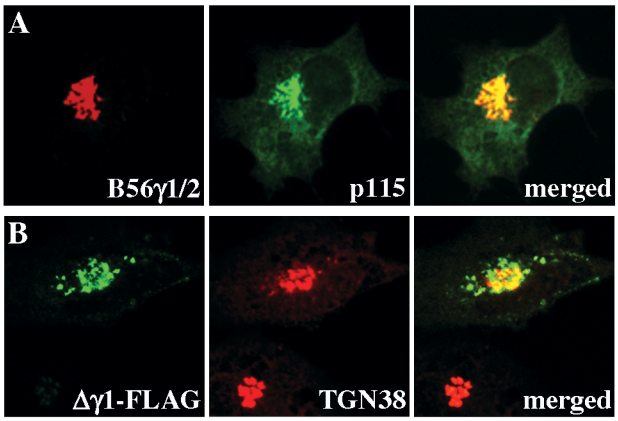

Fig. 4. Localization of the B56γ subunit and Δγ1 at the Golgi complex. **A.** F10 cells were double labeled with an anti-B56γ antibody that recognizes the B56γ1 and γ2 isoforms preferentially (B56γ1/2; left) and an anti-p115 antibody (p115; middle). **B.** NRK cells that express Δγ1-FLAG transiently were double labeled with an anti-FLAG antibody (left) and an anti-TGN38 antibody (middle). The left and middle images were then merged (right).

Given that B56γ1 and Δγ1 localize at the Golgi complex, we wondered whether the expression of Δγ1 could influence Golgi functions. Intracellular vesicle transport is one of the most important functions of the Golgi complex and its efficacy can be quantified by chasing the green fluorescent protein (GFP)-linked vesicular stomatitis virus G protein (VSVG-GFP). This protein is retained in the endoplasmic reticulum (ER) at the restrictive temperature (40 °C), and move out from the ER, through the Golgi, to the plasma membrane when the temperature is reduced to 32 °C (permissive temperature). VSVG-GFP was expressed transiently in NIH3T3$^{\Delta\gamma1}$ and mock-transfected NIH3T3 (NIH3T3^{Vec}) cells and was stored in the ER by keeping the temperature at 40 ºC (Fig. 5). Then the protein was allowed to move out from the ER by shifting the temperature from 40 ºC to 32 ºC. Thirty minutes later, most GFP fluorescence was accumulated in the Golgi complex in both NIH3T3$^{\Delta\gamma1}$ and NIH3T3^{Vec} cells (Fig. 5). It took one more hour for GFP fluorescence to reach the cell periphery in NIH3T3$^{\Delta\gamma1}$ cells while it took two more hours in NIH3T3^{Vec} cells (Fig. 5). This indicates that VSVG-GFP was transported from the Golgi to the plasma membrane faster in NIH3T3$^{\Delta\gamma1}$ cells and suggests that Δγ1 accelerates Golgi-mediated vesicle transport.

Efficient vesicle transport is required for establishment of cell polarity and directional cell

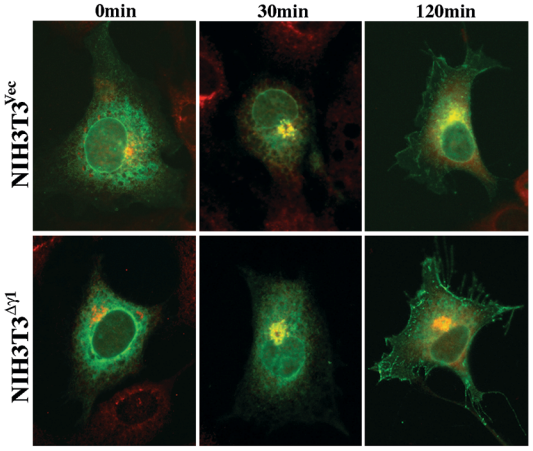

Fig. 5. Accelerated transport of VSVG-GFP from the Golgi area to the plasma membrane in Δγ1-expressing cells. Cells were transfected with a plasmid expressing VSVG-GFP and incubated for 16 hours at 40 °C, during which time the VSVG-GFP accumulated in the ER (0 min). Then the cells were incubated at the permissive temperature (32 °C) for 30 and 120 minutes to allow the VSVG-GFP to be transported to the cell periphery through the Golgi area. Because cells were stained with an anti-p115 antibody (red fluorescence), the Golgi area was colored yellow when VSVG-GFP located at the Golgi area. Note that the cell periphery of NIH3T3$^{\Delta\gamma1}$ cells was decorated with green fluorescence much more strongly than that of NIH3T3^{Vec} cells after 120 minutes of the temperature reduction.

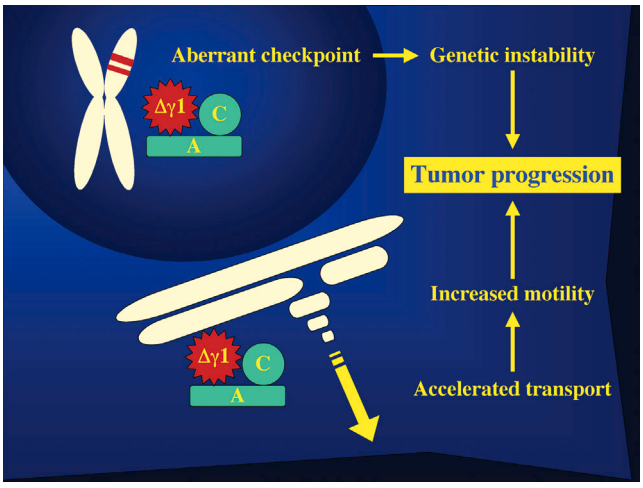

Fig. 6. Two ways how Δγ1 could contribute to malignant progression of melanoma cells. In the cytoplasm, Δγ1 localizes at the Golgi complex as well as B56γ1, and consequently accelerates Golgi-mediated vesicle transport probably by disturbing the function of the B56γ1-containing PP2A holoenzymes. Since efficient delivery of adhesion molecules to the destined sites is necessary for directional migration, expression of Δγ1 may result in enhancement of cell motility. In addition, Δγ1 causes the degradation of γ-irradiation-induced cell-cycle checkpoint integrity. In response to genotoxic stress, cells expressing Δγ1 may acquire more alterations of the genome, some of which aid metastasis.

migration (Bershadsky and Futerman, 1994; Kulkarni et al., 2000). We compared directional migratory ability of NIH3T3$^{\Delta\gamma1}$ cells with that of NIH3T3^{Vec} cells by scratching subconfluent monolayers of these cells (Goodman et al., 1985; Bershadsky and Futerman, 1994). Ten hours after the wounding, NIH3T3$^{\Delta\gamma1}$ cells migrated into the cell-free area from the initial border of the wound 30% faster than NIH3T3^{Vec} cells (Fig. 6). Because the intracellular delivery of adhesion molecules, such as NCAM (Musch et al., 2001), paxillin (Norman et al., 1998) and integrins (Ng et al., 1999; Johanna et al., 2002), to the cell surface is mediated directly by vesicle transport, more efficient vesicle transport may facilitate a more rapid recruitment of adhesion molecules to cell contact sites. Δγ1 is expressed by BL6 cells, a highly metastatic subline, but not by F10 cells, the parental subline. Although further studies are necessary to clarify the relationship between vesicle transport and metastasis, our observations suggest that Δγ1 might contribute to the increased invasive ability of BL6 cells by activating Golgi function. We showed previously that paxillin efficiently localizes to cell adhesion sites at an early stage of NIH3T3$^{\Delta\gamma1}$ cell adhesion. We attributed this phenomenon then to an enhanced phosphorylation of paxillin. Our update observations suggest that accelerated vesicle transport in NIH3T3$^{\Delta\gamma1}$ cells may also contribute to this phenomenon.

Concluding remarks

Originally, we characterized Δγ1 incapable of promoting the dephosphorylation of specific substrates that is normally mediated by the B56γ subunit-containing PP2A holoenzyme (Ito et al., 2000a). Because Δγ1 seems capable of behaving as a targeting subunit, it is likely that dephosphorylation activity provided specifically by the B56γ-containing PP2A holoenzymes may be lowered in cells expressing Δγ1. This notion is supported by a recent study showing that expression of N-terminally truncated B56γ subunits in Drosophila results in phenotypes similar to those produced by loss of function of B56 (Hannus et al., 2002). N-terminally truncated isoforms of the B56γ subunit, including Δγ1, appear to function as dominant-negative mutants that specifically interfere with B56γ subunit-containing PP2A holoenzymes. These mutant isoforms differ clearly from okadaic acid in that their inhibitory actions are in a holoenzyme subtype-specific manner. In fact, Δγ1 accelerates Golgi-mediated vesicle transport while okadaic acid arrests vesicle transport by inducing Golgi fragmentation. This discrepancy can thus be explained probably by the possibility that okadaic acid inhibits all PP2A holoenzymes while Δγ1 interferes only with those containing the B56γ subunit. Mutant isoforms of the PP2A regulatory subunits may become a powerful tool in the field of protein phosphatase studies.

In this review, we showed that Δγ1 has two actions that could facilitate malignant progression of melanoma cells (Fig. 6). To date the mutations resulting in Δγ1 or similarly truncated forms of the B56γ subunit have not been found in human melanoma cells. But, it has been recently reported that expression of the PP2A B56γ gene is markedly suppressed in melanoma cells when compared with benign nevus cells derived from the same patients (Deichmann et al., 2002). Thus, reduced expression of the B56g subunit might contribute to malignant progression of human melanoma cells by perturbing the regulation of cell-cycle checkpoint and Golgi function.

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