A Novel Mechanism for Chaperone-mediated Telomerase Regulation during Prostate Cancer Progression

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ABSTRACT

Telomerase activity has been detected in >85% of all malignant human cancers, including 90% of prostate carcinomas. Because this well-characterized experimental prostate cancer system, we have found that telomerase activity is notably increased (>10-fold) during tumorigenic conversion. Expression profiles of the telomerase components (hTR and hTERT) revealed no substantive changes, which suggests a nontranscriptional mechanism for increased activity. Because the hsp90 chaperone complex functionally associates with telomerase, we investigated that relationship and found that along with telomerase activity, a number of hsp90-related chaperones are markedly elevated during transformation, as well as in advanced prostate carcinomas. Using the nontumorigenic cell protein extract as the source of telomerase, addition of purified chaperone components enhanced reconstitution of telomerase activity, which suggests a novel mechanism of increased telomerase assembly via a hsp90 chaperoning process during prostate cancer progression.

INTRODUCTION

In humans, progressive telomere shortening has been implicated as a cause of cellular senescence (1, 2). Cells capable of bypassing senescence and escaping crisis most often reactivate the enzyme telomerase (3, 4), resulting in stability of telomeric ends and continued cellular proliferation. Most malignant human cancers (>85%) express telomerase (5), which suggests a role for telomerase as a novel and obvious target for cancer diagnostics and therapeutics. Understanding the regulation of telomerase as cells progress toward a cancer-like phenotype is, therefore, critical to determining effective means for targeted cancer therapy.

Normal somatic cells do not express telomerase activity and, therefore, shorten their telomeres with each cell division. The catalytic subunit of telomerase, hTERT,3 has been cloned (6, 7) and has been shown to be capable of restoring telomerase activity by expression in normal cells (8, 9). Stable expression of telomerase in a variety of normal cells devoid of activity results in maintenance of telomere lengths and extension of cellular life span (10, 11), implicating telomere attrition as a major cause of cellular senescence.

Telomerase is a ribonucleoprotein that is minimally comprised of an integral RNA template (hTR) and a reverse transcriptase protein component (hTERT; Refs. 6–8, 12). The molecular size of the telomerase complex has been determined previously with telomerase activity. In addition, Euplotes data indicate that higher-order complexes are more active than the smallest active complex at about Mr550,000 (13), similar to the size suggested for purification of active telomerase from human cells (14). These data suggest that additional proteins or complexes associated with the telomerase holoenzyme are able to modulate its activity. Although there has been identification of a telomerase-associated protein (TEP-1; Refs. 15, 16), it does not appear required for catalysis nor is it exact telomerase-related function known. However, we have recently identified the hsp90 chaperone complex as regulatory elements necessary for telomerase assembly and function both in vitro and in vivo (17). The hsp90 chaperone complex has been shown to facilitate the folding of glucocorticoid receptors as well as other reverse transcriptases from viral origins (18–21). The hsp90 foldosome is minimally composed of hsp90, p23, hsp70, HOP, and hsp40/tdj (18, 19).

Importantly for our study, only about 10% of BPH displays detectable telomerase activity, whereas advanced prostate carcinoma expresses high levels of telomerase (~90%; Ref. 5). Moreover, those rare BPH specimens with telomerase have significantly lower levels of activity than advanced prostate cancers. Using an experimental system for cancer development consisting of human nontumorigenic, tumorigenic, and metastatic prostate epithelial cells derived from the same lineage (22–24), we have observed a significant increase in telomerase activity levels as immortalized prostate epithelial cells progress from the nontumorigenic to tumorigenic phenotype without any observable change in the expression of the telomerase template RNA (hTR) or the catalytic subunit (hTERT). Because transcriptional regulation remains unchanged for the critical telomerase core components, we investigated the role of the hsp90 chaperone complex, the only known set of proteins to associate functionally with telomerase (17). We found that the hsp90-associated chaperones (hsp90, p23, hsp70, and hsp40/tdj) are dramatically increased in parallel with tumorigenic progression, which suggests an enhanced assembly of telomerase as the mechanism for the activity increase. To our knowledge, this is the first report that links the importance of a chaperone-mediated telomerase increase to tumorigenic conversion.

MATERIALS AND METHODS

Cells and Culture Conditions. The parental P69 cell line was immortalized using SV40 T antigen; and tumorigenic/metastatic cell lines (M2182, M2205, and M12) were selected by sequential passaging in athymic nude mice after s.c. injections (Refs. 22–24; Fig. 1). Cells were cultured in defined media (RPMI 1640) supplemented as previously described (22–24). DU145 cells were cultured in DMEM with 10% fetal calf serum (Hyclone, Logan, UT) and 25 μg/ml gentamicin (Life Technologies, Inc., Gaithersburg, MD).

Telomerase Assays. Detection of telomerase activity involves the extension of an oligonucleotide, which serves as the substrate for the telomerase enzyme, followed by PCR amplification of the telomerase products (3, 4). The TRAP-ez telomerase detection kit was used to detect telomerase activity as recommended by the manufacturer (Intergen, Gaithersburg, MD; Ref. 25). After a 30-min telomerase extension at room temperature, samples were subjected to PCR for 27 cycles, followed by electrophoresis on 10% PAGE.
Fig. 1. Summary of the derivation of the SV40 Tag immortalized human prostate cell lines. As described in detail elsewhere (22–24), tumorigenic sublines of the SV40 T antigen immortalized prostate epithelial cells (P69SV40T) were subjected to sequential cycles of in vivo passage in athymic nude mice. The parental P69SV40Tag line is rarely tumorigenic after s.c. or orthotopic injection. The M2182 line is tumorigenic after s.c. injection, whereas M12 is tumorigenic and metastatic after intraprostatic injection.

Gels were visualized and quantitated using a phosphorimager (Molecular Dynamics, Sunnyvale, CA). Relative telomerase activity was measured as the ratio of the telomerase-specific ladder to the 36-bp internal control.

RT-PCR Analysis. Total RNA was isolated by guanidinium thiocyanate, and 2.5 μg of total RNA was annealed with short random oligonucleotides (decamers) and retrotranscribed into cDNA using the first-strand synthesis kit RETROscript (Ambion Inc., Austin, TX). PCR reactions were performed using a fraction of the cDNA reaction. Both hTERT and hTERT were amplified as described previously (6). The amplification of 18S rRNA was performed using a 3:7 mixture of 18S RNA primer pairs and 18S rRNA Competimers (Ambion) for 23–25 cycles. All of the samples were assayed simultaneously to minimize experimental variation. Amplified products were resolved on a 6% PAGE and visualized by staining with ethidium bromide.

Microarray Fabrication. A nonredundant set of 1500 prostate-derived cDNA clones were identified from the Prostate Expression Database (PEDB), from the prostate normal database (PNDB), and from the prostate cancer database (PCDB). Individual cDNA clones were amplified by PCR as template and processed as previously described (27).

Probe Construction and Microarray Hybridization. Total RNA was isolated from P69 and M12 cells using TRIzol (Life Technologies, Inc.). Fluorescence-labeled probes were made from 1 μg of poly(A)+ RNA or 30 μg of total RNA in a reaction volume of 20 μl containing 1 μl of anchored oligo(dT) primer (Amerham-Pharmacacia), 0.05 mM Cy3-dCTP (Amerham-Pharmacacia), 0.05 mM dCTP, 1.0 mM each dGTP, dATP, dTTP, and 200 units Superscript II reverse transcriptase (Life Technologies, Inc.). Reactants were incubated at 42°C for 120 min followed by heating to 94°C for 3 min. Unlabeled RNA was hydrolyzed by the addition of 1 μl of 5 N NaOH and heating to 37°C for 10 min, followed by neutralization. After chromatography (Qiagen), 1 μg of da/dT 12–18 (Amerham-Pharmacacia) and 1 μg of human CotI DNA (Life Technologies, Inc.) were added to the probe, heat denatured, combined with an equal volume of 2x microarray hybridization solution (Amerham-Pharmacacia) and prehybridized at 50°C for 1 h. The mixture was then placed onto a microarray slide with a coverslip and hybridized in a humid chamber at 52°C for 16 h. After washing, the slide was rinsed in distilled water to remove trace salts and dried.

Image Acquisition and Data Analysis. Fluorescence intensities of the immobilized targets were measured using a laser confocal microscope (Molecular Dynamics). Intensity data were integrated at a pixel resolution of 10 μm using ~20 pixels per spot, recorded at 16 bits. For each experiment, each cDNA was represented twice on each slide, and the experiments were performed in duplicate producing four data points per cDNA clone per hybridization probe. Intensity ratios for each cDNA clone hybridization with probes derived from P69 and M12 cells were calculated.

Protein Extraction and Immunoblotting. Cells were harvested at 60–80% confluency using a modified radioimmuno protection assay buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1% aprotinin, and 100 mM DTT). Lysates were clarified by centrifugation at 14,000 × g for 20 min at 4°C. Five to 25 μg of total cellular protein were subjected to 8–12% SDS-PAGE at 150 V for 1.5 h and transferred to nitrocellulose (Hybond; Amersham-Pharmacacia, Buckinghamshire, England) at 100 V for 1 h. Membranes were blocked in PBS with 0.1% Tween 20 (PBS-T) and 5% milk for 1 h, followed by incubation in primary antibody for up to 2 h at room temperature. All of the chaperone antibodies are commercially available from Stressgen (Vancouver, British Columbia) and were used at the following dilutions in PBS-T with 5% milk: hsp90 (AC88, 1:5000); hsp70 (C92F3A-5, 1:5000); p23 (J3, 1:5000); hsp27 (G3.1, 1:2000); hsp40/tdj (Abl, 1:10,000); hsf-1 (SPA-901, 1:10,000); and β-actin (1:5000; from Sigma Chemical Co.). Blots were washed and incubated with secondary antibody (1:5,000 for both mouse and rabbit) conjugated with horseradish peroxidase (Amersham) in PBS-T and 5% milk for 1 h at room temperature. After washing, the signal was detected using the SuperSignal Chemiluminescent Substrate kit (Pierce, Rockford, IL), followed by detection using both autoradiography and digital imaging with the ChemiImager 4400 (Alpha Innotech, San Leandro, CA). Because two antibodies can be used simultaneously to probe a given blot with the standard, β-actin antibody, effective quantitation can be achieved by spot densitometry and determining the ratio of the β-actin signal to that of the specific band of interest.

In Vitro Reconstitution. To assemble telomerase, 5 μl of water-sonicated P69 extract was mixed with water, hsp90/p23, hsp90/p23/hsp70/hsp40, or rabbit reticulocyte lysate in a 10-μl assembly assay and incubated at 30°C for 90 min. To assemble telomerase with purified cofactors, the P69 extract as above was mixed with 500 ng of p23, 750 ng of hsp90, 125 ng of hsp70, 25 ng of HOP (p60), and 25 ng of hsp40/pdj-1 in the presence of 10 mM Tris-HCl, 50 mM KCl, 5 mM MgCl2, and 2 mM DTT (pH 7.5) in a total volume of 10-μl. Of the resulting reconstitution, 2-μl (corresponding to 4,000 cell equivalents) were subjected to the TRAPEze assay.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissues from four cases of BPH and 14 cases of prostate carcinoma were immunohistochemically analyzed for hsp90 and p23 expression. Four-μm tissue sections were deparaffinized in xylene, rehydrated in graded alcohols, and washed in PBS. The tissue sections were microwaved in 10 mM citrate buffer solution for antigen retrieval at pH 6.0 in a thermostresistant container for 12 min and cooled for 20 min (28). Endogenous peroxidase activity was quenched in 0.3% hydrogen peroxide for 10 min. The sections were then incubated overnight at 4°C with either a p23 (1:3000 dilution), hsp90 (1:100 diluted; Ref. 17), or cytomegalovirus (negative control) monoclonal antibody (Mab 8120; 1:1000 dilution; Chemicon International, Temecula, CA). Antibigen-antibody complexes were visualized using a streptavidin-biotin staining technique (Vector Laboratories, Burlingame, CA) according to the manufacturer’s recommendations. Diaminobenzidine (DAB) was used as a chromogen, and hematoxylin as a counterstain.

RESULTS

For our experiments, we have used a human prostate cancer experimental system developed previously (Refs. 22, 24; Fig. 1). Normal prostate epithelial cells taken from a patient after radical prostatectomy were transfected with SV40 large T antigen and selected on the basis of growth (immortalization; Ref. 22). As summarized in Fig. 1, the parental SV40 T antigen-immortalized prostate epithelial cell line, P69SV40Tag, is rarely tumorigenic, but after an extended latent interval, 2 mice of 17 that were given injections did form tumors (M1929 and M2205; 22). From each of these sporadic tumors, cell lines were established in culture and subsequently reinjected into nude mice. One additional tumor-derived, nonmetastatic cell line was cultured (M2182) and again reinjected for an additional round of in vivo selection to obtain metastatic lines, where the tumorigenic and metastatic cell line, M12, was established (24). These cell lines, derived from a single immortalized cell line, provide a system from a defined genetic lineage in which to study the molecular changes that occur during prostate cancer progression.

Telomerase Activity and Telomerase Component Expression Levels in Tumorigenic Prostate Cells. The levels of telomerase activity in the P69 cell line and its derived tumorigenic and metastatic lines were reproducibly elevated >10-fold in the tumorigenic cell lines in at least three independent experiments (Fig. 2A). This dramatic increase in telomerase further indicates the importance of telomerase activation in the transformation process (29). There is very little difference in the telomere lengths in these cells, with the P69 cell line having long and heterogeneous telomere lengths, whereas M12
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Fig. 2. Increased telomerase activity as prostate cells progress toward tumorigenicity without a change in telomerase subunit expression levels. A, representative telomerase assay (TRAP) showing the tumorigenic cell lines (M2205, M2182, and M12) with the nontumorigenic line, P69, using 250- and 1000-cell equivalents. Below each lane, quantitation of each lane, produced by determining the ratio of the telomerase ladder:36-bp internal standard. B, semiquantitative RT-PCR of hTERT mRNA, hTR, and 18S RNA as quantitative control. For each sample, equivalent amounts of RNA were used and amplified for 34 (hTERT), 26 (hTR), or 22 (18S) PCR cycles.

Fig. 3. Substantial increase in hsp90 and p23 levels during tumorigenic conversion. Log phase cells were harvested for Western analysis using antibodies to chaperones p23 and hsp90, as well as the quantitative control actin. Total cellular protein (25 μg) was separated by 12% SDS-PAGE and transferred to nitrocellulose. After blocking, the blot was incubated with primary antibodies for p23 and hsp90, followed by secondary antibody detection using chemiluminescence. The original blot was stripped and reprobed with an antibody to β-actin for semiquantitative analysis.

and M2182 have shorter, more homogeneous lengths (data not shown).

Human telomerase is minimally composed of two associated components, a RNA component (hTR), which serves as the template for telomeric DNA synthesis (12), and a catalytic protein subunit (hTERT), which provides the polymerization function (8). Using a semiquantitative RT-PCR assay, we found no discernible difference for hTR or hTERT mRNA levels when comparing the nontumorigenic P69 cells with the tumorigenic group (M2182, M2205, and M12; Fig. 2B), suggesting a nontranscriptional mechanism for the telomerase increase in this prostate cancer system.

hsp90 Chaperones and Telomerase. The hsp90 chaperone machinery has been shown to be required for functional assembly of human telomerase (17). During the course of microarray analysis of RNA from the P69 and M12 cell lines, the M12 mRNA for hsp90 bound 3-fold higher to hsp90 cDNA targets than that for the P69 mRNA (data not shown). Because there were no substantial differences in the levels of the telomerase components and the microarray data suggested an increase in hsp90 in the tumorigenic line M12, these findings prompted a study of the mechanisms for enhanced telomerase activity other than transcriptional regulation: an increase in the assembly of the telomerase holoenzyme. Consistent with the microarray data, Fig. 3 demonstrates a sizable increase in the expression levels of hsp90 and p23, known telomerase interactors. Relative amounts were determined by comparison with the β-actin internal control and suggest a 5- to 10-fold increase in hsp90 and p23 levels as cells progress to a tumorigenic state (Fig. 3). In addition, we found by Northern analysis that hsp90 mRNA levels were elevated in the tumorigenic cells when compared with the P69 cells (data not shown). To determine the generality of this finding, we tested the DU145 prostate tumor-derived cell line for increased chaperone levels and also found a uniform increase in chaperone levels, consistent with the levels seen among tumorigenic cell lines from P69SV40Tag system (Fig. 3). In fact, it appears that the expression of a number of molecular chaperones was dramatically elevated as cell progress to a tumorigenic state (Fig. 4). In 2–3 independent experiments, the levels of hsp70 and hsp27, and to a lesser extent hsp40, are increased on the order of 2- to 4-fold in the M2182 and M12 tumorigenic cell lines when compared with the nontumorigenic line P69. In addition, hsf-1 was also substantially enhanced in the tumorigenic cell lines (Fig. 4B), suggesting a transcriptional mechanism for the chaperone increase during prostate cancer progression. At this point, it is critical to note the importance of the microarray data, which provided the key evidence to suggest the alternative to telomerase assembly/regulation and the role of chaperones in tumor progression.

To directly address the concept that increased assembly is responsible for the elevated telomerase activity during prostate cancer progression, we established an in vitro assembly reaction using the P69 cell extract as the hTERT/hTR provider. Previous experiments used a rabbit reticulocyte system for expression of hTERT

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and assembly of functional telomerase (17). We showed that telomerase assembly (hence, activity) was increased with the addition of either additional rabbit reticulocyte lysate, which contains an abundance of chaperones, or purified components of the hsp90 chaperone complex to the assembly reaction and that hsp90 and p23 were absolutely required for the assembly process. Therefore, P69 cells were lysed in water by gentle sonication and subjected to a modified *in vitro* assembly reaction using purified chaperone components, as described previously (17). The addition of purified chaperones or rabbit reticulocyte lysate (which contains an abundance of chaperones) provides a reproducible increase in telomerase activity levels (Fig. 5); nevertheless, telomerase activity was not enhanced by the addition of nonspecific protein (data not shown). This suggests that there is a significant amount of unfolded hTERT in the P69 cells, and that supplementing the extract with additional purified chaperones promotes the assembly of active telomerase activity. Taken together, these data indicate that enhanced telomerase assembly, rather than increased expression of telomerase components, is the cause of the elevated telomerase expression in tumorigenic cells.

**Elevated hsp90 and p23 in Malignant Prostate Carcinoma.** We have also found that by immunocytochemistry, hsp90 and p23 are much more abundant in the tumorigenic cell lines (M2182, M2205, and M12) than in the nontumorigenic line (P69; data not shown). Given that information, a series of archival prostate adenocarcinomas were analyzed immunohistochemically for hsp90 and p23 expression. The carcinomas (14 of 14 tested for this study) typically expressed high levels of hsp90 and p23 (Fig. 6, top panels) compared with normal or BPH within the same section (Fig. 6, bottom panels). For both the BPH and prostate carcinoma sections, control antibodies were negative for staining (data not shown). The antibodies for hsp90 and p23 are highly specific as evidenced by the lack of background
observed after Western blotting (see Fig. 3). Whereas secretory epithelial cells in the hyperplastic glands exhibit only weak cytoplasmic staining for p23, basal cells of the glands are consistently intensely stained (Fig. 6). Although telomerase is primarily nuclear and hsp90 and p23 are predominantly cytoplasmic, we observed specific, intense nuclear staining for both hsp90 and p23, which implies that either telomerase assembly occurs in the nucleus or that hsp90 and p23 remain associated with the functional telomerase enzyme. On the basis of our data, we suspect that contrary to other chaperone targets, hsp90 and p23 remain stably bound to active telomerase (30).

**DISCUSSION**

Telomerase reactivation and telomere maintenance appear to be molecular hallmarks of human cancer cells. Understanding the regulation of telomerase during cancer progression is critical for determining the mechanisms of cancer development. Currently, the only known proteins that functionally interact with telomerase are those that comprise the hsp90 molecular chaperone complex (17). Chaperones have been shown to be absolutely required for the assembly of human telomerase both in vitro and in vivo. The experimental system of human prostate cancer progression used for the present study clearly demonstrates the importance of telomerase expression as a cellular requirement for tumorigenic conversion and transformation (29). This prostate cancer progression model has proven a powerful tool for studying the molecular mechanisms related to malignant tumor development, especially as it relates to the sequence of events during tumor progression. Our results conclusively show that although expression of the minimal core telomerase components was unchanged, telomerase activity itself was dramatically increased as prostate cancer cells progressed to a tumorigenic state. Taken together, these data indicate a lack of transcriptional regulation and suggest an alternative mechanism for the observed telomerase activity increase.

The hsp90-related molecular chaperones have been shown to be elevated in a variety of cancers (reviewed in Ref. 31). We show that hsp90 levels were elevated in the tumorigenic M12 cells compared with the rarely tumorigenic P69 cells by microarray analysis. We also found that a number of chaperone-related proteins, including hsp70, p23, hsp40, hsp27, and hsf-1, are dramatically increased during prostate cancer progression, not only in this experimental system, but in an alternative prostate tumor-derived cell line and in primary prostate cancers as well. Therefore, these data suggested that increased telomerase assembly, rather than expression of telomerase components, occurs during tumorigenic conversion. This was confirmed by enhanced telomerase activity after the addition of purified chaperones in an in vitro P69 reconstitution system, which suggests that a significant portion of the telomerase is unfolded or unassembled because of inadequate chaperone expression. To our knowledge, this is the first report showing an increased chaperone-mediated assembly of telomerase as a mechanism that contributes to tumorigenic conversion.

The increased chaperone levels observed during prostate cancer progression may function not only to assemble telomerase but also to stabilize the hTERT protein and prevent degradation. With some chaperone proteins expressed in limiting amounts, certain other chaperones (specifically hsp70) are capable of targeting chaperone-associated proteins for ubiquitin-mediated degradation (32). Importantly, we have shown that inactive hTERT, but not active telomerase, is associated with hsp70. Thus, in the absence of adequate hsp90 and/or p23, P69 hTERT may be targeted for hsp70-dependent degradation. Even so, our results indicate that there is a significant fraction of unfolded/inactive hTERT in P69 cells, given the fact that we observe increased telomerase activity after the addition of purified chaperone components using lysates from the low telomerase P69 cells. Together, these data suggest that there is substantial unfolded telomerase in extracted P69 cells and chaperones are the limiting components for increased telomerase assembly and activity.

It is important to distinguish our study from that of others regarding the hsp90, hsp70, and hsf-1 (33). We show substantial differences in hsp90 and hsp70 expression between nontumorigenic and tumorigenic cells, in which Hoang et al. (33) compare tumorigenic-but-nonmetastatic to metastatic cells and find no changes in hsp90 or hsp70 levels. However, they do find that the transcription factor hsf-1 is elevated in metastatic PC-3 cells, whereas we find no enhancement of hsf-1 between M2182 (tumorigenic) and M12 (metastatic). The reason for this discrepancy in hsf-1 expression levels is unclear but may reflect differences in the culture conditions or the experimental systems themselves.

The hsp90 foldosome is minimally composed of hsp90, p23, hsp70, HOP, and hsp40/ycj (18, 19). For glucocorticoid receptors, effective ligand binding requires stabilization of the receptor, and purified chaperone proteins mediate the formation of active receptor, establishing sufficient protein for a functional complex. Whereas hsp90 and p23 appear associated with the receptor, hsp70, HOP, and hsp40 remain essential for the ATP-dependent hsp90/p23-receptor association. Purified versions of each of the 5-foldosome proteins were able to mediate the assembly of functional telomerase in vitro (17). Here, we show that increased assembly of human telomerase in extracts from the nontumorigenic P69 cells is mediated by the addition of purified chaperones. It is important to note that whereas expression of the hTERT transcriptionally may not change, the association with chaperones may stabilize the protein and provide for an increased half-life of hTERT, allowing for the enhanced telomerase activity observed during progression. However, because we found that the addition of purified chaperones to a P69 extract enhanced activity, this suggests that chaperone-mediated hTERT stability only partially contributes to the telomerase enhancement observed, and increased assembly of telomerase is occurring.

The question still remains as to how chaperone-mediated enhancement of telomerase contributes to tumorigenesis. One possibility is that the tumorigenic cell lines shorten their telomeres more rapidly than the nontumorigenic cells and maintenance at such a short length (our tumorigenic cells have shorter telomeres than nontumorigenic cells) requires increased telomerase activity, providing cells with the ability to continuously proliferate. Alternatively, it may be possible that the telomerase increase is only an effect of the elevated chaperone levels, and that the chaperone increase contributes to the transformation process. Currently, each of these options is under investigation to understand the role of telomerase and chaperones in tumorigenic transformation and how this interaction contributes to the maintenance of a tumorigenic phenotype.

The mechanism for chaperone increase during prostate cancer transformation may be in part at the transcriptional level, as indicated by some of the data presented here. First, the M12 subline exhibits a reproducible increase in hsp90 mRNA expression using both microarray and Northern analyses. Second, the tumorigenic cells have elevated protein levels of hsf-1, which is known to have binding sites at the promoters of hsp90, hsp70, and hsp27 (34). Thus, the increase in chaperones and telomerase assembly may be a novel mechanism for prostate cancer transformation that is ultimately mediated by hsf-1. In addition, progression of prostate cancer may require a ubiquitous increase in chaperone levels and protein folding. Because the hsp90 chaperone complex affects a number of cellular targets, it is likely that the overall up-regulation of chaperones during transformation will increase
the activity and function of many other signaling and growth factor pathways. Even so, telomerase, as an hsp90 target, remains a critical component in the tumorigenic conversion process. We have shown in an experimental model of cancer progression, and in prostate carcinoma tissues, that the hsp90-associated chaperones are consistently up-regulated in malignant epithelial cells. Thus, we believe that detection of elevated levels of hsp90/p23/hsp70, as well as telomerase, may be useful for cancer diagnostics. In addition, increased chaperone levels and/or telomerase may have prognostic value in predicting prostate cancer or tumorigenic/meta-static potential in less aggressive disease. Further study into the Gleason’s score and tumor grade will be critical to our understanding of the chaperone/telomerase association during prostate cancer progression.

Hsp90 blocking agents, such as geldanamycin and radicicol, have been touted as anticancer compounds and are currently in clinical trials (35). Geldanamycin is a benzoquinone ansamycin that blocks the association of p23 by associating with the hsp90 molecule in the ATP binding pocket. Although geldanamycin does not prevent hsp90 from associating with its target, it does prevent the folding or assembly of those targets that would normally occur in the presence of ATP and p23. Without question, the hsp90 machinery (hsp90 and/or p23) is a potential target for gene- or chemotherapy, especially knowing that hsp90-associated chaperones are up-regulated as cancer cells progress. Because telomerase is a target for hsp90-mediated assembly of functional enzyme, inhibition of hsp90 function would likely inhibit telomerase and potentially prevent cancer progression. We have previously shown that geldanamycin blocks telomerase assembly in vitro and in vivo (17). However, hsp90 has numerous targets within a given cell, and blanket treatment with hsp90 inhibitors may yield nonspecific effects on both normal and cancer cells. Therefore, inhibition of telomerase assembly as a method for adjuvant cancer therapy using hsp90 as a target needs to be a highly specific and efficient process. Considering all of this type of anti-telomerase therapy suggests that combinational treatments will be most effective and that further investigation into the mechanisms underlying the telomerase assembly process is required.

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