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Award Number: W81XWH-04-1-0580

TITLE: Regulation of hTERT Expression and Function in Newly Immortalized p53 (+) Human Mammary Epithelial Cell Lines

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REPORT DATE: June 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-06-2005	Annual	1 Jun 2004 – 31 May 2005

4. TITLE AND SUBTITLE

Regulation of hTERT Expression and Function in Newly Immortalized p53 (+) Human Mammary Epithelial Cell Lines

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0580

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Martha R. Stampfer, Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California
Lawrence Berkeley National Laboratory
Berkeley, CA 94720

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Original contains color plates: ALL DTIC reproductions will be in black and white

14. ABSTRACT – SEE ATTACHED PAGE

15. SUBJECT TERMS
hTERT/Telomerase; Immortal Transformation; p53; Genomic Instability; Human Mammary Cells; Early Stage Carcinogenesis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	8	

Abstract

Telomerase is reactivated in almost all human breast cancers; the immortal potential conferred by telomerase is thought to be crucial for malignant progression. Loss of telomeric protection usually leads to widespread genomic instability. This proposal is to study telomerase reactivation and telomere protection in newly immortalized human mammary epithelial cells (HMEC) that retain wild type p53 function, and to determine if these cells may be especially sensitive to therapies that target telomerase activity and telomere protection. Prior work showed that p53 can suppress most, but not all, telomerase expression in newly immortal p53+ HMEC lines until telomeres become extremely short, when an unknown mechanism (termed conversion) relieves this repression. The cyclin-dependent kinase inhibitor p57 may protect cells with these critically shortened telomeres by inhibiting growth until there is sufficient telomerase reactivation to protect the telomeric ends. Our current aims are: (1) Test whether the low telomerase activity, as well as the elevation of p57 expression seen in newly immortal p53+ HMEC, suppress the genomic instability seen prior to immortalization, and if inhibition of telomerase activity and p57 function might efficiently kill these cells. (2) Determine how p53 regulates telomerase activity in newly immortal HMEC lines.

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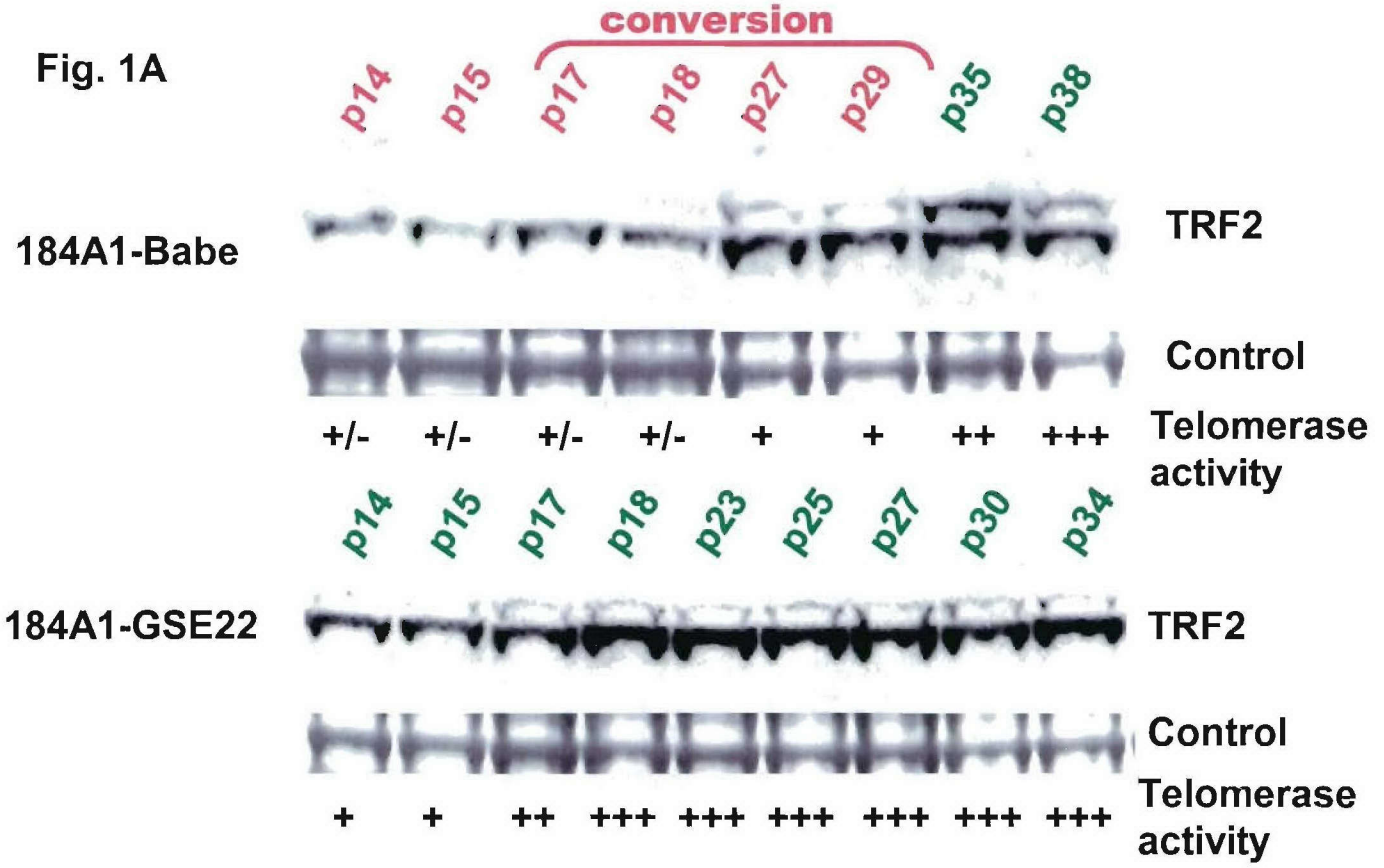
INTRODUCTION:

Acquisition of an immortal potential is considered crucial for human carcinogenesis, in order for a single cell to accumulate the multiple errors necessary for malignancy. In human carcinomas, attaining immortality is associated with reactivation of telomerase activity, which maintains the telomeric ends. Loss of telomeric protection usually leads to telomeric associations and genomic instability. This proposal is designed to study the mechanisms involved in telomerase reactivation and telomere protection in newly immortalized human mammary epithelial cells (HMEC) that retain wild type p53 function, and to determine if these cells may be especially sensitive to killing by agents that target telomerase activity and telomere protection. Prior work showed that p53 can suppress most, but not all, telomerase expression in newly immortal p53+ HMEC lines until telomeres become extremely short (mean TRF $\leq$3 kb), when an unknown mechanism (termed conversion) relieves this repression (Stampfer et al, 2003, Stampfer et al, 1997). Preliminary data also suggested that the cyclin-dependent kinase inhibitor p57, whose expression is rapidly elevated when mean TRF declines to $\leq$ 3kb (Nijjar et al., 1999), may protect these critically shortened telomeres by inhibiting growth until there is sufficient telomerase reactivation to protect the telomeric ends. Our current aims are: (1) Test whether the very low levels of hTERT present in newly immortal p53+ HMEC lines preferentially maintain the shortest telomere ends until the conversion associated relief of p53-mediated suppression of telomerase activity occurs. Additionally, test whether this low telomerase activity, as well as the elevation of p57 expression, suppress the genomic instability seen prior to immortalization (Romanov et al, 2001, Chin et al., 2004), and if inhibition of telomerase activity and p57 function might efficiently kill these cells. (2) Determine how p53 regulates telomerase activity in newly immortal HMEC lines.

BODY:

Work on this new grant was delayed due to a delay in obtaining the human use approval for this project. This approval as obtained from our local IRB on 5/31/05 and approval from DOD was acknowledged on 6/6/05. As a consequence, we were unable to proceed with new studies on this grant until this month. Key personnel on this grant did however work with a postdoctoral fellow, Ekaterina Bassett, who has independent postdoctoral funding on a related topic, thereby providing some data relevant to specific aim 2.

Our prior work indicated that p53 function in our cultured HMEC could be inactivated using a genetic suppressor element (GSE22) that acts as a dominant negative inhibitor of p53 tetramer formation (Stampfer et al. 2003). When GSE22 was transduced into a newly immortalized p53+ line, 184A1, levels of hTERT expression and telomerase activity rapidly increased, and telomere length was stabilized (Stampfer et al, 2003). We now looked at expression of telomere-associated proteins, to see if there were any changes in their expression or localization that might correlate with immortalization or conversion. No significant changes were seen in expression of TRF1, Tin2, Rap1, Ku70, Ku80, or WRN when comparing finite, newly immortal (pre-conversion; very low telomerase), and fully immortal (post-conversion, high telomerase) HMEC. However, increased expression of TRF2 was observed by Western blotting in the fully immortal lines (as well as many breast tumor cell lines) compared to both pre-conversion and finite HMEC (Nijjar et al, 2005). Immunoflourescence (IF) assays showed that this increased expression was associated with a minority population (15-30%) that had a larger cell and nuclear size. To determine if this change in TRF2 expression correlated with conversion and telomerase reactivation, the same population of 184A1 transduced with GSE22 that had been studied for telomerase expression was now assayed for TRF2 protein expression by Western blotting and protein localization by IF. As shown in Figure 1A and B, increased TRF2 expression correlated with increased telomerase expression during conversion. Since overexpression of hTERT in pre-conversion 184A1 by itself did not result in increased TRF2 expression (Nijjar et al, 2005), this change must be due not simply to the expression of telomerase, but as a consequence of other changes occurring during conversion and endogenous reactivation of telomerase activity. We postulate that conversion in newly immortal p53+ HMEC is triggered by a change in telomere structure when the mean TRF has declined to $\leq$3 kb. The observed alteration in TRF2 expression and localization may provide a clue to the nature of the changes incurred when these cells enter conversion/reactivate telomerase.



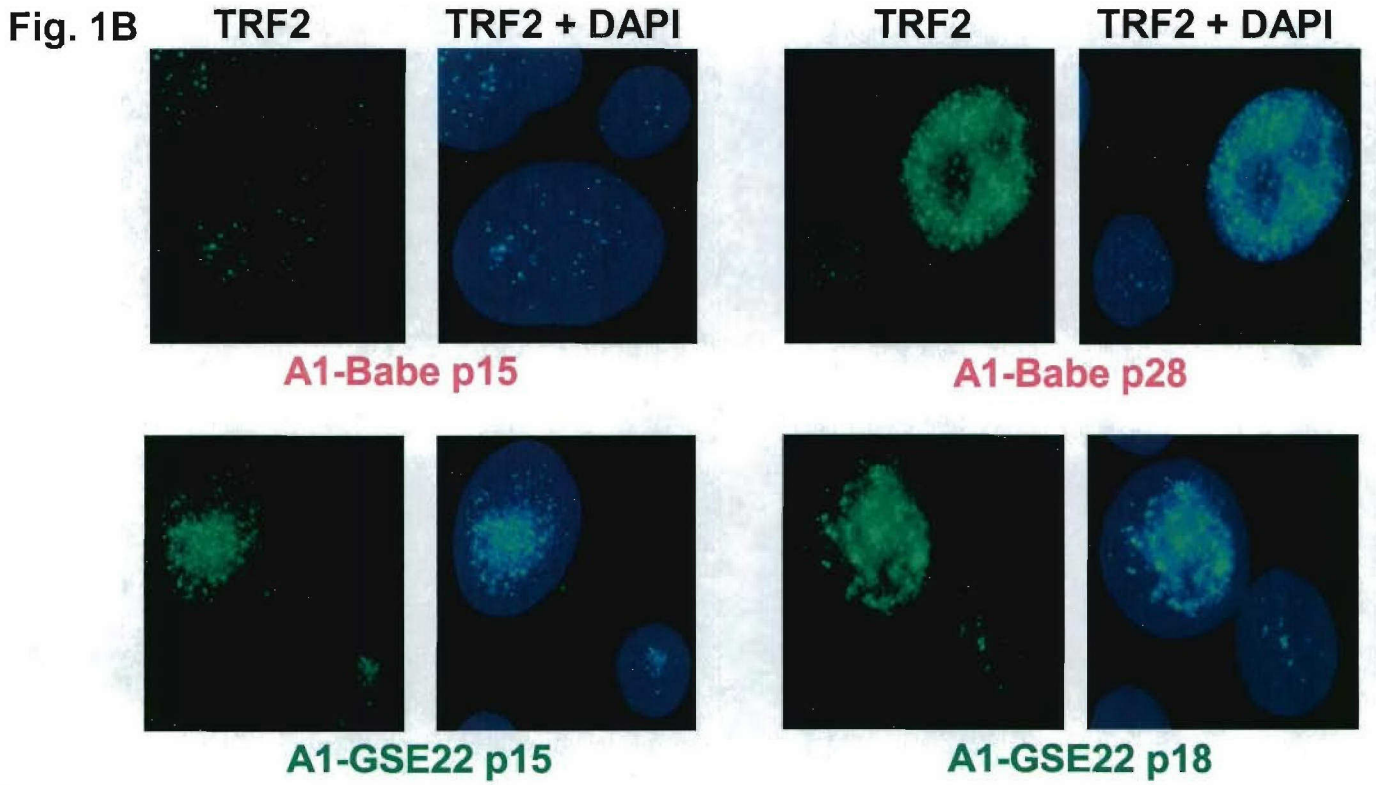


Figure 1. Inactivation of p53 in Immortal HMEC Causes an Immediate Increase in TRF2 Protein Similar to the Increase in Telomerase Expression. (A) Western blotting; (B) Immunoflorescence.

KEY RESEARCH ACCOMPLISHMENTS:

• Demonstrated that changes in the telomere-associated protein TRF2 correlate with telomerase reactivation in newly immortal p53+ HMEC.

REPORTABLE OUTCOMES:

N/A

CONCLUSIONS:

Telomerase is reactivated in almost all human breast cancers; the immortal potential conferred by telomerase is thought to be crucial for malignant progression. Expression of hTERT, the catalytic subunit of human telomerase, is the rate-limiting component of telomerase activity.

Our research has uncovered novel steps in the immortalization of p53+ HMEC involving telomerase repression and reactivation. These studies have shown that newly immortal p53+ HMEC possess exceedingly short telomeres, yet are protected from the widespread genomic instability that could lead to cell death. Our current proposal seeks to expand upon this data, both in terms of understanding the basic mechanisms regulating telomerase expression in these cells, and to determine if such cells could be especially vulnerable to therapies that target telomerase activity, and other factors protecting against catastrophic genomic instability.

Due to our delay in starting, our results thus far are limited to gaining further evidence of significant phenotypic changes in TRF2 expression and localization associated with telomerase reactivation and conversion in newly immortal p53+ HMEC (specific aim2).

We are currently planning to expand these studies on the mechanisms of p53-dependent telomerase repression and reactivation by: (1) determining how quickly inactivation of p53 results in hTERT reactivation. The timing of reactivation will help distinguish between signaling pathways immediately downstream of p53, or alternative pathways which are affected by p53, but are not located immediately downstream of it. (2) Investigation of how transcriptional regulators Sp1, Sp3, and p21 may be involved in p53-mediated regulation of the hTERT promoter in the HMEC undergoing conversion. (3) Determine whether critically short telomeres play a role in telomerase derepression during conversion through activating intracellular signaling pathways involved in DNA damage recognition and repair.

We will also begin our studies addressing specific aim 1 by testing the effects of telomerase inhibitors and siRNA to p57 on the growth properties and genomic stability of newly immortal p53+ HMEC.

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APPENDICES:

N/A