ARPN Journal of Science and Technology

http://www.ejournalofscience.org

Mini Review Telomerase-A Biomarker in the Carcinogenesis and Diagnosis

[1] Malti Narwaria, [2] Archana Shrivastav, [3] B.R. Shrivastav

[1] College of Life Sciences, Cancer hospital and research Institute, Gwalior, Madhya Pradesh, India.
[2] Professor and Head, Deptt. Of Microbiology, College of Life Sciences,
Cancer hospital and research Institute, Gwalior, Madhya Pradesh, India.
[3] Director, Cancer hospital and research Institute, Gwalior, Madhya Pradesh, India

ABSTRACT

Telomerase is an attractive cancer target as appear to be required in essentially all tumors for immortalization of a subset of cells, including cancer stem cells. Moreover differences in telomerase expression and telomere length between normal and tumor tissues suggest that targeting telomerase would relatively safe. In normal adults, telomerase activation mainly presents in tissue stem cells and progenitor cells giving them unlimited growth potential. Cells immortalized in vitro, on the other hand, express telomerase and maintain their telomeres. Telomerase activity has also been detected in the large majority of tumors from a variety of cancers [1]. Telomerase expression is a potentially important marker of high-grade cervical dysplasia and squamous cell carcinoma (SCC).Ectopic expression of telomerase reverse transcriptase (TERT), the protein catalytic subunit of telomerase, can reconstitute telomerase activity, lengthen telomeres, and bypass both senescence and crisis, endowing human fibroblasts with immortal proliferative properties. Telomerase is a fundamental marker of euplastic transformation that is widely expressed in both premalignant intraepithelial lesions and in most malignant lesions of the uterine cervix [41], and therefore, is a potential element in the carcinogenesis and diagnosis.

Keywords: *Telomerase, Immortality, Euplastic.*

1. INTRODUCTION

The process of cancer formation called as tumorigensis. Tumorigensis is a multistep process involving genetic mutation leads to malignant phenotype. Six essential genetic alterations manifesting themselves in cell physiology contribute to the malignant transformation of a cell. There are six main steps whose alterations contribute to malignant transformation commonly refereed as hallmarks of cancer. These can be:

- Self-sufficiency in growth signals.
- Insensitivity to growth inhibitory signals.
- Evasion of apoptosis.
- Limitless explicative potential.
- Sustained angiogenesis.
- Tissue invasion and metastasis [2].

Telomerase is a reverse transcriptase having its own RNA component which acts as a template and adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions. Telomeres are very end region of eukaryotic chromosome which prevents constant loss of important DNA segment from chromosome ends .The existence of a compensatory shortening of telomere (telomerase) mechanism was first predicted by Soviet biologist Alexey Olovnikov in 1973[2], who also suggested the telomere hypothesis of aging and the telomere's connections to cancer. Telomerase was discovered by Carol W. Greider and Elizabeth Blackburn in 1984 in the ciliate Tetrahymena [4]. The protein composition of human telomerase was identified in 2007 by Scott Cohen and his team at the Children's Medical Research Institute in Australia. It consists of two molecules each of human telomerase reverse transcriptase (TERT), telomerase RNA (TR or TERC), and dyskerin (DKC1) [5]. The genes of telomerase subunits, which are TERT, TERC, DKC1,and TEP1etc, are located on the different chromosomes in human genome. Human TERT gene (hTERT) is translated into a protein of 1132 amino acids. During cell division progeny cells after certain period of time reach a Hay flick limit in normal conditions due to lack of telomerase [6]. When cells lost their senesce, become immortal and the presence of telomerase triggers uncontrolled proliferation of cells and each dividing cell can replace the lost bit of DNA are associated with a variety of premature aging syndromes with short telomeres [7]. These include Werner syndrome, Ataxia elangiectasia, Ataxia-telangiectasia like disorder, Bloom syndrome, Fanconi anemia and Nijmegen breakage syndrome. To explore telomerase in aging first its molecular characterization is essential which is still under investigation. Cancer arises due to a common theme: activation of TERT, loss of p53 pathway function, loss of pRb pathway function, activation of the Ras or myc proto-oncogenes, and aberration of the PP2A protein

ARPN Journal of Science and Technology

phosphates. That is to say, the cell has an activated telomerase, eliminating the process of death by chromosome instability or loss, absence of apoptosis-induction pathways, and continued activation of mitosis. Of the tumors that have not activated TERT [12], most have found a separate pathway to maintain telomere length termed ALT (Alternative Lengthening of Telomeres [8].The exact mechanism behind telomere maintenance in the ALT pathway has not been elucidated, but likely involves multiple recombination events at the telomere As we know that telomerase is necessary for the immortality of so many different cancer types, it is thought to be a potential drug target. If a drug can be used to turn off telomerase in cancer cells, the above process of telomere-shortening will resume—telomere length will be lost as the cells continue to divide, mutations will occur, and cell stability will decrease. Experimental drug and vaccine therapies targeting active telomerase have been tested in mouse models, and some have now entered early clinical trials. Geron Corporation is currently conducting four human clinical trials involving telomerase inhibition and telomerase vaccination. Merck, as a licensee of Geron, has recent approval of an IND for one vaccine type. The vaccine platform is being tested using different approaches. However, a variety of caveats, including the presence of the ALT pathway, complicate such therapies [9].

2. WHEN IS TELOMERASE ACTIVATED DURING CANCER DEVELOPMENT?

Requirement of telomerase for immortality and ultimately to malignancy have been proven by different scientist invitro and invivo. .Activity of telomerase is mostly absent in normal human somatic cells but become activated during tumor genesis and cellular immortalization. Still mechanism behind telomerase activation is not fully understood may include telomerase catalytic subunit gene (hTERT) amplification and trans-activation of the hTERT promoter by the oncogene product. Many cells can immortalize as they restore ectopic expression of hTERT. (42) Telomerase activity has been detected in both germ line cells and in the developing embryo. However, activity is turned off in most somatic cells of the neonate although; low levels of activity seem to persist in regenerative tissues. Telomerase is found to be reactivated or up regulated in the majority of cancers. If this activation occurs early during tumor genesis, then it may be a useful marker for cancer detection and early diagnosis. If, on the other hand, it is a late event, then it may be better suited for staging or prognostic purposes. Specific cancers in which there is evidence that activation occurs early include the following: bladder, liver, colorectal head and neck, lung, cervix, kidney prostate, and thyroid. In these tumor types, neither the presence or absence of activity, nor the levels of activity correlate with markers known to have prognostic value. Cancers, in which there does appear to be a correlation between activity status and tumor grade, stage, prognostic factors, or patient survival, include: meningiomas, neuroblastomas, non-Hodgkin's lymphomas, and certain leukemia's. Conflicting results have been reported for gastric and breast cancers. In the breast, activity has been found in pre-malignant lesions and most studies fail to find any link between activity and known prognostic factors [10].

3. SENSITIVITY AND OTHER ASSAY METHODS

To detect telomerase with specificity and sensitivity variety types techniques are available .Some cell types including male germ cells, activated lymphocytes, and stem cell populations continue to express telomerase at reduced levels [11,12,13,]. However, the majority of immortalized cells express telomerase, and one of the most exciting findings in recent years is the identification of telomerase activity in greater than 85% of human cancers, encompassing a broad range of cancer types, with little or no activity in most normal somatic tissues [14]. In 1989, telomerase activity was detected by Morin in an immortalized human cancer cell line (HeLa), and later by Counter during the in vitro immortalization of human embryonic kidney cells whose telomere lengths were then seen to stabilize [15,16]. Finally, in 1994, telomerase activity was detected in human ovarian and B-cell cancers, confirming Olovnikov's prediction of an "anti-marginotomy" mechanism at work in malignant cells [17, 18]. Unfortunately, the primer extension assay used up to this point for detecting telomerase activity required large amounts of tissue, making widespread application of the method difficult. Then, in December of 1994, Kim et al. published their Telomeric Repeat Amplification Protocol (TRAP) which featured an improved tissue lysis method and PCR amplification of the telomerase-extended oligonucleotide substrate [15]. These improvements resulted in a 10,000-fold increase in assay sensitivity, thereby allowing the use of very small (milligram or less) amounts of tissue. Since then, there has been a flurry of investigations in which telomerase activity has been assayed in normal, benign lesions, pre-malignant, and cancerous samples of many human tissue types, using either the original or one of various modified versions of the TRAP assay as there is no currently agreed upon standard method for measuring or quantitating activity.

The assay has recently been modified to allow for the quantitative assessment of telomerase using

ARPN Journal of Science and Technology

enzyme-linked immunosorbent assay technology. Studies using the TRAP assay have shown that expression of telomerase is strictly regulated, at least in human tissues. Telomerase activity is essential for embryogenesis but is repressed upon tissue differentiation during development such that telomerase is absent from birth in most somatic tissues [19]. With the advancement of more new techniques like Microarray technology is an orderly arrangement of known or unknown DNA samples attached to a solid support [20], Biosensor detection devices [21], Electrochemical impedance spectroscopy[22] ,along with a number of telomerase based strategies are used in cancer therapy to improve diagnosis and prognosis value.

4. SPECIFICITY OF TELOMERASE ACTIVITY IN CANCER

For the measurement of telomerase activity to be useful clinically, either its presence must be restricted to malignant tissues or activity levels must be quantitatively distinguishable from those present in normal and benign tissues. Early surveys of telomerase activity in human material found only rare instances of active telomerase in non-malignant tissues compared to the vast majority of cancers which were discovered to be telomerase positive [23]. These initial observations supported the hypothesis that cancer cells were primarily telomerase-positive and immortal while their normal somatic cell counterparts were telomerase-negative and mortal. This is in keeping with the observed telomere shortening and limited life spans of non-euplastic cells. Since then however, the widespread use of sensitive PCR-based assays has resulted in the detection of activity in increasing numbers of some types of both normal and benign tissues. The fact that some normal tissues possess telomerase activity was not unanticipated. Research group hypothesized that the stem cell pools of regenerative tissues such as the hematopoietic system, the lining of the gut, and the epidermis of the skin, would likely require active telomerase in order to sustain the large amount of cell turnover observed in such systems [24]. They tested this hypothesis in male rodent secondary sex-accessory tissues which can be experimentally cycled to grow, involutes, and regrow by manipulation of serum testosterone levels. In the prostate for example, lowering testosterone causes glandular involution and the loss of over 90% of prostate cells. The small residual prostate and seminal vesicles that remain following testosterone withdrawal are highly enriched for stem cells and display high levels of telomerase activity. Furthermore, activity is rapidly down-regulated in conjunction with glandular regeneration which occurs following the re-introduction of.testosterone[25].

The first indication that normal human tissues possessed active telomerase came from the discovery that peripheral blood leukocytes and bone marrow cells from normal donors had detectable levels of enzyme activity [26, 27, and 28]. This was surprising because it was known from previous studies that these stem cell populations exhibited telomere shortening. Interestingly, in the hematopoietic system, telomerase activity was not restricted to the most primitive stem cells but was also found in mature T-cells, B-cells, macrophages, and granulocytes [26, 28, and 29]. Although the activity detected was generally much weaker than that found in established lines of immortal tissue culture cells or in malignant cells. However, in vitro studies have shown that activity can be up-regulated by a number of activating mutagenic stimuli including: phytohemagglutinin in T-cells, pokeweed mitogen in B-cells, phorbol ester/Ca-ionophore combinations, and various anti-cell surface receptor antibodies [22-28]. In addition to bone marrow and peripheral blood cells, normal and benign lymph nodes have also been shown to be telomerase-positive, with activity localized to proliferating B-cells in the germinal centers of secondary follicles [31,32] . Moreover, recent studies have shown that activity clearly exists in normal skin and cervical epidermis [32-35], hair follicles [32, 36], the endometrial of the uterus [37-40], vascular endothelial cells [41], as well as the lower portions of the crypts within the colonic mucosa [34]. All of these tissues are undergoing continuous cell turnover and, where examined, telomerase activity appears restricted to regions harboring stem cells and proliferating amplifying cell pools, while this activity is apparently lost in other zones in conjunction with terminal differentiation. The overall prevalence of telomerase activity in normal and benign euplastic solid tissues are both 27%, significantly greater than in earlier reports. However, lymph nodes, which are nearly always found to be positive, represent greater than one-half of the benign and approximately one-third of the normal positive samples making up this average .The bulk of the remaining positive cases come from tissues with renewal and regenerative properties such as the skin, uterus, and colon. If these three sources, along with lymph nodes, are excluded from consideration in the overall averages then the incidence of telomerase-positive cells in normal solid tissues drops to 6%. On the whole it seems that, in addition to the aforementioned telomerase activity found in normal blood and bone marrow, activity is also commonly detected in non-cancerous samples of other actively cycling tissues. This background activity may compromise the clinical value of telomerase assays in these regenerating tissue types. If activity could be

reliably quantities it might be possible to set reasonable cut off limits that would differentiate between cancerous and non-cancerous samples [24].

5. CONCLUSION

Although more than 25 years have passed since the discovery of telomerase (Greider and Blackburn, 1985), this remarkably complex enzyme still retains many mysteries. Telomerase genes are now characterized in a number of organisms, making comparative studies and enabling reconstitution and complementation studies. Multiple levels of telomerase regulation have been discovered and this knowledge is being exploited in designing novel strategies of anticancer therapy or telomerase targeted drug therapy [1]. Characterization of the newly cloned protein subunits as well as illumination of the enzymatic mechanism of telomerase will hopefully provide useful new targets for our continuing war on cancer [11]. Role of telomerase in immortalization has been clearly established (Hahn et al., 1999), its value as a cancer inhibitory target is yet to established and has not fully been proven by clinical studies that report an actual significant therapeutic benefit in cancer patients. The prospect of adding telomerase-based therapies to the growing list of new anticancer products is promising, but what are the advantages and limitations of different approaches, and which patients are the most likely to respond? Still has to be answered. . Molecular staging using markers such as telomerase activity in combination with other molecular markers will be highly useful.

ACKNOWLEDGEMENTS

I am grateful to Dr.Archana Shrivastav for encouragement, for critical reading of the manuscript and also thankful to Dr. B.R. Shrivastav for providing me resources without which this review would have not been possible.

REFRENCES

[1] Eva Sykorva and Jar Fajkust, Structure-function relationship in telomerasegene.Biol.Cell, 2009, 101,375-392.

[2] Joharia Azhar, Tumorogenesis; The dual role of Telomerase.Iranian J. of Path, 2009, 4(2), 51-58.

[3] Olovnikov AM, "A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotide's and biological significance of the phenomenon". J. Theor. Biol. 1973, 41 (1), 181–90.

[4] Greider CW, Blackburn EH, "Identification of a specific telomere terminal transferee's activity in Tetrahymena extracts". Cell, 1985, 43 (2 Pt 1), 405–13.

[5] Cohen S, Graham M, Lovrecz G, Bache N, Robinson P, Reddel R. "Protein composition of catalytically active human telomerase from immortal cells". Science 2007,315 (5820), 1850–3.

[6] Hayflick L, Moorhead PS,"The serial cultivation of human diploid cell strains". Exp Cell Res, 1967, 25,585–621.

[7] Blasco MA, "Telomeres and human disease: ageing, cancer and beyond". Nat. Rev. Genet., 2005, 6 (8): 611–22.

[8] Henson JD, Neumann AA, Yeager TR, Reddel RR. , "Alternative lengthening of telomeres in mammalian cells". Oncogene, 2002, 21 (4), 598–610.

[9] Vulliamy T, Marrone A, Goldman F, Dearlove A, Bessler M, Mason PJ, Dokal I. ,"The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita". Nature, 2001,413 (6854), 432–5.

[10] A. K. Meeker1 and D. S. Coffey1, 2 Telomerase: a Promising Marker of Biological Immortality of Germ, Stem, and Cancer Cells. A Review 1997

[11] Blackburn, E. H. , Nature,1991,350, 569-572.

[12] Harley, C. B., Vaziri, H., Counter, C. M., and Allsopp, R. C. , Exp. Gerontol,1992,27, 375-382.

[13] Yoshida, K., Sugino, T., Tahara, H., Woodman, A., Bolodeoku, J., Nargund, V., Fellows, G., Goodison, S., Tahara, E., and Tarin, D, Cancer, 1997, 362-369.

[14] Morin, G., Cell, 1989, 59, 521-529.

[15] Counter, C., Avilion, A., LeFeuvre, C., Stewart, N., Greider, C., Harley, C., and Bacchetti, S. EMBO J, 1992, 11, 1921-1929.

[16] Counter, C. M., Hirte, H. W., Bacchetti, S., and Harley, C. B.Proc. Natl. Acad. Sci., 1994, USA, 91, 2900-2904.

[17] Nilsson, P., Mehle, C., Remes, K., and Roos, G. Oncogene, 1994, 9, 3043-3048.

[18] Kim, N. W., Piatyszek, M. A., Prowse, K. R., Harley, C. B., West, M. D., Ho, P. L., Coviello, G. M., Wright, W. E., Weinrich, S. L., and Shay, J. W. Science, 1994, 266, 2011-2015.

[19] Colin S Cooper Applications of microarray technologyin breast cancer research. Breast Cancer Res, 2001, 3,806-813.

[20] Eliona Kulla and Evgeny Katz .Biosensor Techniqueas used for determination of Telomerase Activity in Cancer Cells. Sensors, 2008, 8,347-369

[21] Weiqiang Yang et al. Label,"free detection of telomerase activity in HeLa cells using electrochemical impedance spectroscopy". Chem.Commun, 2011, 47, 3129-3131.

[22] Rhyu, M. S. ,J. Natl. Cancer Inst., 1995,87, 884-894.

[23] Tsao, J. I., Zhao, Y. L., Lukas, J., Yang, X. W., Shah, A., Press, M., and Shibata, D. Clin. Cancer Res., 1997, 3, 627-631.

[24] Meeker, A. K., Somerfield, H. J., and Coffey, D. S. (1996) Endocrinology, 1996, 137, 5743-5746.

[25] Broccoli, D., Young, J. W., and de Lange, T. Proc. Natl. Acad. Sci. USA, 1995, 92, 9082-9086.

[26] Counter, C. M., Gupta, J., Harley, C. B., Leber, B., and Bacchetti, S. Blood, 1995, 85, 2315-2320.

[27] Hiyama, K., Hirai, Y., Kyoizumi, S., Akiyama, M., Hiyama, E., Piatyszek, M. A., Shay, J. W., Ishioka, S., and Yamakido, M. J. Immunol., 1995, 155, 3711-3715.

[28] Chiu, C. P., Dragowska, W., Kim, N. W., Vaziri, H., Yui, J., Thomas, T. E., Harley, C. B., and Lansdorp, P. M. Stem Cells, 1996, 14, 239-248.

[29] Yashima, K., Piatyszek, M. A., Saboorian, H. M., Virmani, A. K., Brown, D., Shay, J. W., and Gazdar, A. F. J. Clin. Pathol. 1997, 50, 110-117.

[30] Ohyashiki, J. H., Ohyashiki, K., Iwama, H., Hayashi, S., Toyama, K., and Shay, J. W. (1997) Clin. Cancer Res., 1997, 3, 619-625.

[31] Taylor, R. S., Ramirez, R. D., Ogoshi, M., Chaffins, M., Piatyszek, M. A., and Shay, J. W. (1996) J. Invest. Dermatol. 1996,106, 759-765.

[32] Ueda, M., Ouhtit, A., Bito, T., Nakazawa, K., Lubbe, J., Ichihashi, M., Yamasaki, H., and Nakazawa, H. Cancer Res.,1997, 57, 370-374.

[33] Yasumoto, S., Kunimura, C., Kikuchi, K., Tahara, H., Ohji, H., Yamamoto, H., Ide, T., and Utakoji, T. Oncogene, 1996, 13, 433-439.

[34] Harle-Bachor, C., and Boukamp, P. Proc. Natl. Acad. Sci. USA, 1996, 93, 6476-6481.

[35] Ramirez, R. D., Wright, W. E., Shay, J. W., and Taylor, R. S. J. Invest. Dermatol. 1997, 108, 113-117.

[36] Brien, T., Kallakury, B., Lowry, C., Ambros, R., Muraca, P., Malfetano, J., and Ross, J. Cancer Res.,1997, 57, 2760-2764.

[37] Kyo, S., Takakura, M., Kohama, T., and Inoue, M. Cancer Res., 1997, 57, 610-614. 37

[38] Saito, T., Schneider, A., Martel, N., Mizumoto, H., Bulgaymoerschel, M., Kudo, R., and Nakazawa, H. Biochem. Biophys. Res. Commun., 1997, 231, 610-614.

[39] Hsiao, R., Sharma, H. W., Ramakrishnan, S., Keith, E., and Narayanan, R. Anticancer Res., 1997, 17, 827-832.

[40] Broccoli, D., Young, J. W., and de Lange, T. Proc. Natl. Acad. Sci. USA, 1995, 92, 9082-9086.

[41] Elke A Jarboe1, Kai-Li Liaw .Analysis of telomerase as a diagnostic biomarker of cervical dysplasia and carcinoma. Oncogene, 2002, 21, 664 ± 673

[42] Hahn WC. Meverson M. Telomerase activation, cellular immortalization and cancer. Ann Med 2001, 33(2), 123-9