TELOMERES AND MELANOMA

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Telomeres are the end points of chromosomes and they tend to shorten each mitotic doubling and are often the life limiting factor in cell lifetimes. Several researchers have discovered in a high percentage of melanomas a changed gene which creates an excess of TERT the protein that ensures Telomere survival. We examine this finding and consider some consequences.

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1 INTRODUCTION

Telomeres are those ends of DNA which have the tendency to be lost each time a cell reproduces leading eventually to a loss of function. Cancer on the other hand may have mastered the loss of sections of the telomeres and thus may have an ability to prolong their life to many reproductions, namely unlimited. There has been significant interest in targeting telomeres and especially the related enzyme, telomerase, to control cancer cells. In a recent pair of papers the authors have focused on this process in melanomas and especially on UV activation.

The authors have discovered somatic mutations in TERT genes which are used to produce Tert and control the Telomeres during cell reproduction. In addition they authors argue that these mutations result from UV radiation.

In this paper we review the basics of Telomeres and then consider the results of the work regarding its influence in melanoma.

It is important to note, as we do herein, that there have been ongoing discoveries of somatic gene mutations found in melanoma over the past decade. There are ligands, receptors, extracellular matrix elements, cofactors from benign cells, internal pathway breaks, transcription factors, miRNAs, and the list goes on. Each time the Press, all too often, with the cooperation of the researchers, makes this most recent discovery a sine qua non. However at best each discovery is but one more step in putting a very complex process in context.

As we have previously, we use extensive primary source references to make our points. Thus this paper provides a window to what we currently understand about this specific issue.

2 TELOMERES

The focus on telomeres and cancer has been an area of active interest for almost two decades. As Shay et al (2012) state:

To grow indefinitely, human cancer cells must counteract the progressive loss of telomeric DNA that universally accompanies cell division. To do this, about 85 to 90% of cancers use telomerase, an enzyme that synthesizes the tandem 5′-TTAGGG-3′ hexanucleotide repeats of telomeric DNA by reverse transcription using its own RNA subunit as a template. Because telomerase is not expressed in most normal human cells, telomerase inhibition is considered an almost universal oncology target, and several clinical trials are under way

The above focuses on the critical importance of telomerase. Before continuing it is worth reviewing the telomere. As Shay and Wright state:

Telomeres are tracts of repetitive DNA (TTAGGG/ AATCCC for human telomeres) that protect chromosomes from degradation and loss of essential genes, and allow the cell to distinguish between double-strand breaks and natural chromosome ends. Human telomeres at birth contain 15–20-kilobase pairs of the repetitive sequence TTAGGG followed by a 3′ single-strand overhang on the G-rich strand, which is believed to be inserted within the double-stranded region to give a lariat-like structure called a t-loop.

Telomeres progressively shorten in most human cells with increased age, and telomere length in almost all middle-aged human tissues is approximately half that of the new born length. Telomere-specific proteins (such as protection of telomeres-1 (POT1), telomeric repeat-binding factor-1 (TRF1) and TRF2) bind directly to the single- and double-strand telomere regions to form a complex, providing a cap over the ends of the chromosomes that protects chromosome termini from degradation, recombination and end-joining reactions.

The authors further state that telomeres are somewhat maintained in humans via the use of telomerase as follows:

Telomere length is maintained by a balance between processes that lengthen telomeres, such as the activity of the cellular ribonucleoprotein enzyme complex telomerase, and processes that shorten telomeres, such as incomplete synthesis of the lagging DNA strand and end processing events. Telomerase stabilizes telomere length by adding TTAGGG repeats onto the telomeric ends of the chromosomes, thereby compensating for the continued erosion of telomeres that occurs in its absence.

Human telomerase contains two essential components, a telomerase reverse transcriptase catalytic subunit (hTERT) and a functional telomerase RNA *(hTR, also known as TERC…*

Other earlier authors such as Campisi et al state:

Telomeres are the repetitive DNA sequences and specialized proteins that form the distinctive structure that caps the ends of linear chromosomes. Telomeres allow cells to distinguish the chromosome ends from double strand DNA breaks. The telomeric structure prevents the degradation or fusion of chromosome ends, and thus is essential for maintaining the integrity and stability of eukaryotic genomes. In addition and perhaps less widely appreciated, telomeres may also indirectly influence gene expression.

The length, structure and organization of telomeres are regulated by a host of telomere-associated proteins, and can be influenced by basic cellular processes such as cell proliferation, differentiation, and DNA damage. In mammalian cells, telomere length and/or telomere structure have been linked to both cancer and aging. Here, we briefly review what is known about mammalian telomeres and the proteins that associate with them, and discuss the cellular and organismal consequences of telomere dysfunction and the evidence that cells with dysfunctional telomeres can contribute to cancer and aging phenotypes.

Thus the Telomere and its limiting characteristics is a natural target for investigation in cancer research.

3 CURRENT CONTRIBUTION

We now move to the two papers which were the focus of this paper. We first examine how they were handled in the press and we will then proceed to examining them first hand.

Let us first start with what was as reported in the Harvard Gazette where we have[1]:

Two mutations that collectively occur in 71 percent of malignant melanoma tumors have been discovered in what scientists call the "dark matter" of the cancer genome, where cancer-related mutations haven't been previously found….

This non-coding DNA, much of which was previously dismissed as "junk," accounts for 99 percent of a cell's genome. A large number of oncogenic mutations in cancer have been identified in the past several decades, but all have been found within the actual genetic blueprints for proteins….

"In addition, this represents the discovery of two of the most prevalent melanoma gene mutations. Considered as a whole, these two TERT promoter mutations are even more common than BRAF mutations in melanoma. Altogether, this discovery could cause us to think more creatively about the possible benefits of targeting TERT in cancer treatment or prevention," Garraway said.

The mutations affect a promoter region — a stretch of DNA code that regulates the expression of a gene — adjacent to the TERT gene. TERT contains the recipe for making telomerase reverse transcriptase, an enzyme that can make cells virtually immortal, and is often found overexpressed in cancer cells. A promoter region of DNA controls the rate of a gene's transcription — the copying of its DNA recipe into a message used by the cell to manufacture a protein….

The researchers said the same mutations are present in cell lines from some other malignancies, and that preliminary evidence showed they might be unusually common in bladder and liver cancers. They also noted that the discovery of these important mutations in DNA previously not linked to cancer-causing alterations highlights the value of whole-genome searches of tumor DNA.

This report details one of the two papers. TERT is the gene which allows for potential immortality of the Telomeres. It repairs the ends as they break and if there is enough of it around then the cell can become immortal. Now the essence of this discovery is that a promoter region adjacent to TERT has been mutated so that TERT is turned on all the time, almost. Thus there is an overabundance of TERT and in turn Telomeres never really shorten, and the cell lives each time it goes through mitosis.

Another report on Science 2.0 states[2]:

[1] http://news.harvard.edu/gazette/story/2013/01/mutations-drive-malignant-melanoma/

They analyzed the genomes of family members and found an identical mutation in the gene for telomerase, an enzyme often called 'immortality enzyme', in all persons studied. Telomerase protects the ends of chromosomes from being lost in the process of cell division and, thus, prevents that the cell ages and dies. The inherited gene mutation leads to the formation of a binding site for protein factors in the controlling region of the telomerase gene, causing it to become overactive. As a result, mutated cells overproduce telomerase and hence become virtually immortal.

This finding prompted the scientists to also look for mutated telomerase genes in non-inherited (sporadic) melanoma, which is much more common than the familial variant. In most of the tissue samples of melanomas of all stages they found alterations in the telomerase gene switch, which the researchers clearly identified as typical consequences of sun exposure. Even though these mutations were not identical to those found in the melanoma family, they had the same effect: overactive telomerase…

This is also confirmed by the surprising incidence of this alteration: The telomerase gene is the most frequently mutated gene in melanoma. "This is something we hadn't expected, because malignant melanoma has been genetically analyzed thoroughly. But this mutation always seems to have been overlooked," says Kumar.

It should be noted in the above the reference to sun exposure. The argument is that the telomerase change is a direct consequence of the UV exposure. We will focus on that observation later. The “overlooked” nature of this gene and its product is also of issue in that many researchers have examined telomerase extensively so frankly it is not truly new, even as a target for control.

[2] http://www.science20.com/news_articles/familial_gene_mutation_immortalizes_malignant_melanoma-101871

4 TERT

Before continuing it is worth a quick summary of TERT, the telomerase that maintains the telomere. TERT is located at 5p15.33. From NCBI we have[3]:

Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene, and an RNA component which serves as a template for the telomere repeat. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis.

Studies in mouse suggest that telomerase also participates in chromosomal repair, since de novo synthesis of telomere repeats may occur at double-stranded breaks. Alternatively spliced variants encoding different isoforms of telomerase reverse transcriptase have been identified; the full-length sequence of some variants has not been determined. Alternative splicing at this locus is thought to be one mechanism of regulation of telomerase activity.

The observation can be made that if we do not have adequate TERT then the Telomere ends decay and ultimately the cell line dies off. This is the typical case. Therefore take a malignant melanoma cell. If it has in its pathways and receptors been activated to mitotic duplication then if the TERT is inadequate then the Telomere ends get cut shorter each time it goes through mitosis and at some point it just stops.

For example, and this is just for exemplar purposes only, we have a malignant melanocyte, then it goes through mitosis say 10,000 times but each time it would lose a piece of the Telomere until they are all gone, then th cell cannot go again. But if there is an overabundance of TERT, then the TERT resupplies what may be lost and this cell has no way of stopping, at least due to this factor.

[3] http://www.ncbi.nlm.nih.gov/gene/7015

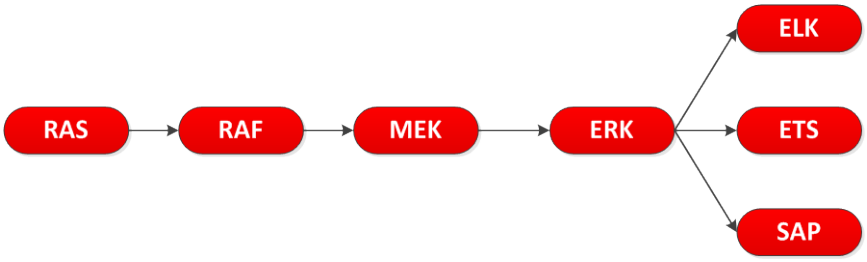
5 ETS

The ETS family of genes is positive or negative regulators of gene expression. They can up or down regulate expression. They are named for the initial gene discovered, the E26 Transforming Sequence, where E26 was the oncogene v-ets characterized in 1986 of an avian transforming virus called E26. It is also called the erythroblast transforming specific family, as discussed by Zong et al.

The ETS family is a large family of over 20 such genes, and we will focus on ERG specifically. The Table below is from Watson et al.

	Subgroup	Name	Unigene Name	Alternative Names	Locus	Size
1	ETS	ETS1	ETS1		11q23.3	441
2		ETS2	ETS2		21q22.3	469
3	ERG	ERG2	ERG		21q22.3	462
4		FLI1	FLI1	ERGB	11q24.1-q24.3	452
5		FEV	FEV		2q36	238
6	PEA3	PEA3	ETV4	E1AF, PEAS3	17q21	462
7		ERM	ETV5		3q28	510
8		ER81	ETV1		7p21.3	458
9	ETV	ER71	ETV2	ETSRP71	19q13.12	370
10	TCF	ELK1	ELK1		Xp11.2	428
11		SAP1	ELK4		1q32	431
12		NET	ELK3	SAP2, ERP	12q23	407
13	GABP	GABP α	GABPA	E4TF1	21q21.3	454
14	ELF1	ELF1	ELF1		13q13	619
15		NERF	ELF2	NERF1, NERF2, EU32	4q28	581
16		MEF	ELF4	ELFR	Xq26	663
17	SPI1	SPI1	SPI1	PU.1, SFPI1, SPI-A	11p11.2	264
18		SPIB	SPIB		19q13.3-q13.4	262
19		SPIC	SPIC		12q23.2	248
20	TEL	TEL	ETV6		12p13	452
21		TEL2	ETV7	TEL-B	6p21	264
22	ERF	ERF	ERF		19q13	548
23		PE-1	ETV3	METS	1q21-q23	250
24	PDEF	PDEF	SPDEF		6p21.3	335
25	ESE	ESE1	ELF3	ESX, JEN, ERT, EPR1	1q32.2	371
26		ESE2	ELF5		11p13-p12	255
27		ESE3	EHF	ESEJ	11p12	300

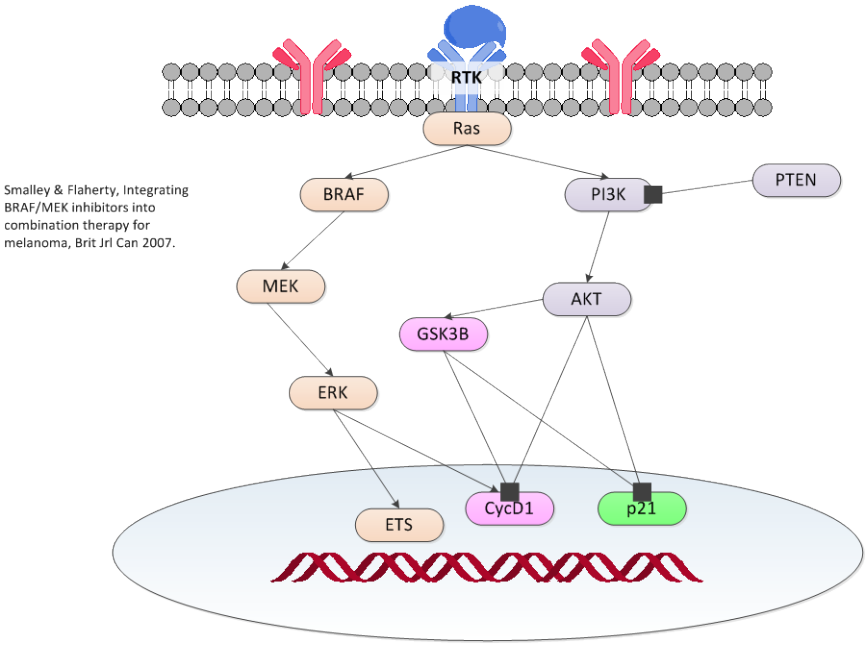
The ERG gene was first presented in the paper by Shyam and Reddy et al in 1987. There the authors identified it and set it in the ETS family. From Weinberg, we see that the ETS are transcription factors driven by the RAS/RAF pathway along with other such factors.



ETS also plays a significant role in the process. We briefly review that as well. ETS is located at 11q23.3. From NCBI we have[4]:

This gene encodes a member of the ETS family of transcription factors, which are defined by the presence of a conserved ETS DNA-binding domain that recognizes the core consensus DNA sequence GGAA/T in target genes. These proteins function either as transcriptional activators or repressors of numerous genes, and are involved in stem cell development, cell senescence and death, and tumorigenesis. Alternatively spliced transcript variants encoding different isoforms have been described for this gene

From Smalley and Flaherty we have the following pathway for ETS:



The mutations we discuss here are somewhat new and they are present in a relatively large number of samples, at least percentage wise. We know that ETS has transcription control and we

[4] http://www.ncbi.nlm.nih.gov/gene/2113

can see from above the relationship to BRAF as well. Thus there are many points of loss of control in a melanoma cell. Specifically, as Chudnovsky et al note[5]:

Multiple genetic alterations occur in melanoma, a lethal skin malignancy of increasing incidence. These include mutations that activate Ras and two of its effector cascades, Raf and phosphoinositide 3-kinase (PI3K). Induction of Ras and Raf can be caused by active N-Ras and B-Raf mutants as well as by gene amplification. Activation of PI3K pathway components occurs by PTEN loss and by AKT3 amplification.

Melanomas also commonly show impairment of the p16 (INK4A)-CDK4-Rb and ARF-HDM2-p53 tumor suppressor pathways. CDKN2A mutations can produce p16(INK4A) and ARF protein loss. Rb bypass can also occur through activating CDK4 mutations as well as by CDK4 amplification. In addition to ARF deletion, p53 pathway disruption can result from dominant negative TP53 mutations. TERT amplification also occurs in melanoma.

The extent to which these mutations can induce human melanocytic neoplasia is unknown. Here we characterize pathways sufficient to generate human melanocytic neoplasia and show that genetically altered human tissue facilitates functional analysis of mutations observed in human tumors.

[5] http://www.ncbi.nlm.nih.gov/pubmed/15951821?dopt=Abstract

6 THE RESULTS

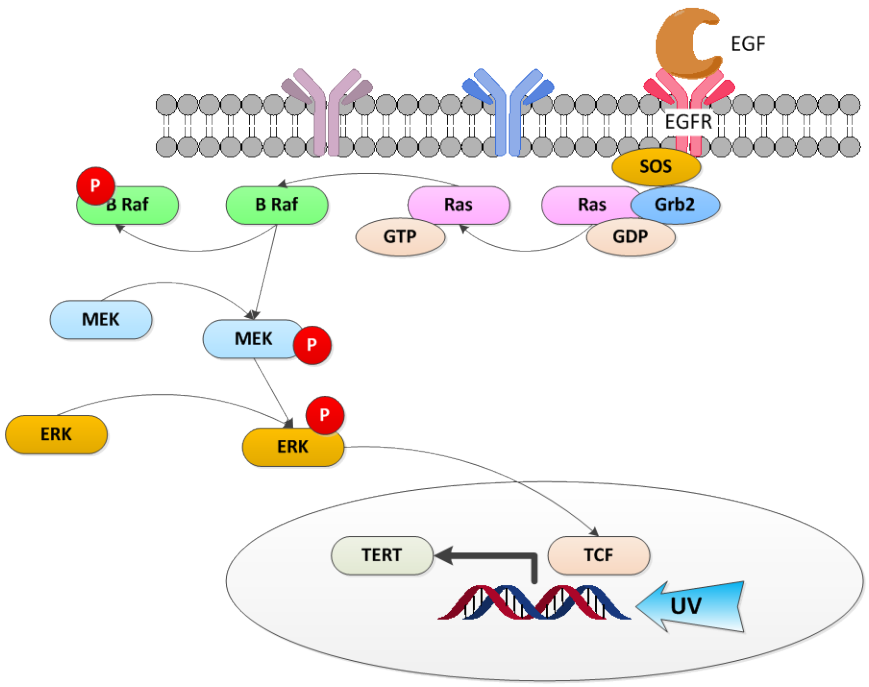
Now we will examine the two recent papers as published in Sciencexpress in early 2013. We start with the paper by Horn et al and then proceed to the second paper.

As Horn et al state:

Cutaneous melanoma occurs in both familial and sporadic forms. We investigated a melanoma-prone family through linkage analysis and high-throughput sequencing and identified a disease-segregating germ line mutation in the promoter of the telomerase reverse transcriptase (TERT) gene, which encodes the catalytic subunit of telomerase. The mutation creates a new binding motif for Ets/TCF transcription factors near the transcription start and in reporter gene assays, caused up to 2-fold increase in transcription.

We then screened the TERT promoter in sporadic melanoma and observed recurrent UV signature somatic mutations in 125/168 (74%) of human cell lines derived from metastatic melanomas, corresponding metastatic tumor tissues (45/53, 85%) and in 25/77 (33%) primary melanomas. The majority of those mutations occurred at two positions in the TERT promoter and also generated binding motifs for ETS/TCF transcription factors.

Horn et al conjecture the following pathway:



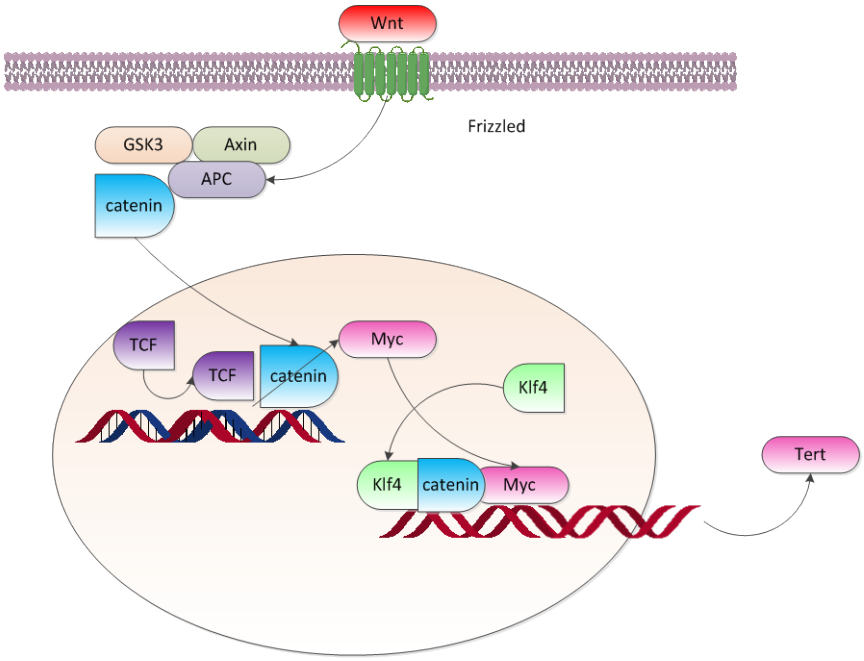
As Huang et al state:

Systematic sequencing of human cancer genomes has identified many recurrent mutations in the protein coding regions of genes but rarely in gene regulatory regions. Here we describe two

independent mutations within the core promoter of TERT, the gene coding for the catalytic subunit of telomerase, which collectively occur in 50 of 70 (71%) of melanomas examined.

These mutations generate de novo consensus binding motifs for ETS transcription factors, and in reporter assays the mutations increased transcriptional activity from the TERT promoter by 2 to 4-fold. Examination of 150 cancer cell lines derived from diverse tumor types revealed the same mutations in 24 cases (16%), with preliminary evidence of elevated frequency in bladder and hepatocellular cancer cells. Thus, somatic mutations in regulatory regions of the genome may represent an important tumorigenic mechanism.

We have discussed before the Wnt pathway connection to TERT as well. As shown below we have discussed this option as well.



This has been discussed by Hoffmeyer as well as by Greider. As Greider states:

Recent studies have proposed that the Wnt pathway is linked to TERT in a quite different way. Constitutive overexpression of TERT in mice activates the Wnt pathway, suggesting that TERT may also function as a transcription factor. Although one study did not observe Wnt pathway activation in response to TERT overexpression, other studies have raised questions about the physiological relevance of the constitutive overexpression of TERT.

Deletion of TERT in mice does not affect expression of target genes in the Wnt pathway, nor give rise to the cellular phenotypes that loss of Wnt signaling induces, indicating that TERT regulation of Wnt signaling may be limited to situations where TERT is overexpressed.

It is reasonable to propose that Wnt regulates TERT given that Wnt signaling plays an essential role in stem cell self-renewal and that TERT is needed for the long-term growth of stem cells. TERT regulation seems to require not one, but two master transcriptional regulators to assure

that there is neither too much, which may allow the growth of cancer cells, nor too little, which might lead to stem cell failure. The finding by Hoffmeyer et al. that both β-catenin and Klf4 are required to activate TERT expression puts the horse (Wnt) before the cart (TERT) and provides a foundation for linking telomerase levels and self-renewal.

Thus TERT regulation is truly a complex process. We have examined the impact of Wnt on melanoma previously. This recent work is on mutations on TERT genes yet we also must consider the influence of Wnt as well.

7 OBSERVATIONS

This discovery leads to several observations of note:

1. One could have imagined something of this happening with Telomeres. It would almost be necessary to allow ongoing uncontrolled mitotic activity. Thus, despite the fact that there is no surprise here we do have a specific target, namely the activator of TERT.

2. Melanoma, as most other cancers, has a multiplicity of changes to genes. There are ligands, receptors, pathway elements, transcription factors, and the telomere issues as well. It is clear that no single factor is the dominant one as of yet. BRAF as a target works for a while and then there is a work around. Thus cancer is an evolving process, and one which may be highly adaptive.

3. A Conjecture: As we have learned more and more as to aberrant genes and their products, as well as miRNAs, and their effects, one could envision several uses of malignancy profiling. We consider that in two steps.

Step 1: Profiling a Specific Patient at Various Locations. As shown below we consider a specific patient and then profile gene expression as a function of distance from the site of initiation, if such was possible. Then we can see how various aberrant genes are being expressed over the distances measure from the source. One would suspect that distance must be measured in some normalized manner but we leave that as an exercise for the student at this time. This gives us a profile for a specific patient, perhaps one for developing therapeutics.

Specific Patient at Different Locations From Source

Gene	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
G1	7	6	6	5	5	5	5	4	4	4	3	3	3	2	2	2	1	1	1
G2	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
G3	6	6	5	5	4	3	2	1	1	1	1	1	1	1	1	1	1	1	1
G4	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
G5	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
G6	6	6	6	5	5	4	4	3	3	2	2	1	1	1	1	1	1	1	1
G7	7	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
G8	7	4	4	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G9	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
G10	8	7	6	6	6	5	5	5	5	5	4	4	3	3	3	3	3	2	1
G11	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
G12	5	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
G13	6	5	4	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
G14	7	7	6	6	6	6	5	5	4	4	3	3	3	2	2	2	2	2	2
G15	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Step 2: The Same Location but across a Large Pool of Patients: Again we look now at the same distance from the source, perhaps at the same time, again an exercise for the student, and we get profiles of the expression of aberrant genes. This allows us to understand the between patient differences.

		Different Patients at Specific Location from Source																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Gene	G1	4	3	4	4	3	2	4	5	5	6	6	4	3	2	3	4	4	2	1
	G2	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
	G3	1	3	4	5	2	2	4	3	2	3	4	5	3	2	5	2	3	4	5
	G4	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
	G5	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
	G6	3	4	5	5	6	2	3	5	5	5	5	7	6	8	8	6	8	8	8
	G7	2	5	4	2	3	4	5	2	2	4	3	2	3	4	5	3	2	5	2
	G8	1	3	2	2	1	1	1	1	1	2	3	2	4	3	3	2	1	3	2
	G9	3	5	7	7	7	7	3	5	8	8	8	5	6	7	7	7	7	4	6
	G10	5	2	3	4	5	5	5	5	6	6	6	6	6	6	4	4	5	3	5
	G11	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
	G12	4	5	6	6	5	7	7	3	4	5	5	6	6	7	6	7	6	5	5
	G13	1	5	3	3	4	6	5	5	5	4	3	2	2	1	5	4	4	3	4
	G14	5	7	8	8	6	7	7	8	8	8	7	7	9	8	8	9	7	8	8
	G15	1	2	2	2	1	1	2	2	2	2	1	1	1	2	2	2	3	2	3

3. Is Seventy Enough? The study did an analysis on 70 lesions. Perhaps that is not enough. Furthermore based upon our previous comments perhaps a correlative study is demanded as well, by patient and by distance.

4. One of the problems I see is the continually hyping of the results as if this is finally the right answer. Anyone even slightly familiar with the field will understand that each input is vital but assembling them in a cohesive whole is essential. The systems approach is the sine qua non, but that cannot be done without the continual bench work required to understand the details.

For example in an article in the Boston Globe the reporter states[6]:

Now scientists working independently in Boston and Germany have made a surprising discovery: a set of genetic mutations found in most melanomas, the deadliest skin cancer. The presence of these mutations in the vast majority of tumors studied suggests that the researchers may have stumbled upon a fundamental mechanism involved in a hallmark trait of cancer cells—their ability to live forever—that could one day be targeted by drugs.

Outside researchers said the work, published online Thursday in the journal Science Express, is exciting because the conclusion is the opposite of what many exhaustive studies of cancers have shown.

[6] http://www.boston.com/news/science/blogs/science-in-mind/2013/01/24/boston-researchers-discover-mutations-that-underlie-melanoma-junk-dna/mNIYVavGfVsvstVj5eNfzO/blog.html

In reality as we have discussed, it was imperative that the Telomeres be preserved in metastasis. Millions of rapid mitotic changes in a stem cell must survive and that means keeping Telomeres and that means lots of TERT. Somehow the conclusion was logical, consistent and not at all unexpected especially given what else has been found in the past decade.

The article continues:

Both teams zeroed in on mutations in a part of the genome called a promoter, which acts like a volume knob on a stereo to control gene activity. The gene that the promoter controlled happened to be one that has long been of interest in cancer because it creates part of an enzyme called telomerase, which enables cancer cells to continue to divide indefinitely as one of its key jobs. Still, it wasn't easy for the researchers to convince themselves that what they found, underlying more than two-thirds of melanoma cases, was real.

One would expect this and if one looks at say the miRNA discoveries, they all add up to what controls the ultimate expression of mitotic survival.

5. Theraputics: Can we expect therapeutics from this understanding? Good question. Kinase inhibitors are now well understood, one could in theory build an inhibitor here as well. Is this the target, another target, necessary, helpful, we can only guess. Yet the above Conjecture may allow for the development of a therapeutic profiling plan for melanoma and other malignancies.

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