LY6 and Prognostic Markers

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LY6 are a class of RNA that have recently been assessed as prognostic of aggressive cancers. They are integral in the immune response functions. The authors of a recent paper also allege relationships to cancer stem cells, CTCs, and we examine that issue as well.

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1 INTRODUCTION

There is a never ending collection of putative gene markers for diagnostic and prognostic value. A recent one from the staff at Georgetown University argues for the prognostic value of a set of genes on 8q24, the LY6 genes. They content that the overexpression of these genes are prognostic for a poor outcome. They argue that these markers have value for a broad spectrum of cancers including prostate.

As noted in Eureka[1]:

An examination of 130 gene expression studies in 10 solid cancers has found that when any of four related genes is overexpressed, patients have much worse outcomes, including reduced survival.

Researchers from Georgetown Lombardi Comprehensive Cancer Center say their study, published Feb. 3 (2016) in Oncotarget, shows that this Ly6 family of genes allows cancer cells to act like cancer stem cells -- which keep dividing and growing without pause.

"These are remarkable findings. We believe this family of genes produces cancer that easily metastasizes, is drug resistant and very difficult to destroy," says the study's senior investigator, Geeta Upadhyay, PhD, research assistant professor of oncology at Georgetown Lombardi.

Upadhyay and her collaborators are currently working on novel agents that can inhibit Ly6 gene expression.

Upadhyay's research was initially based on Sca1, a mouse gene investigators use to check for the presence of cancer stem cells in animals. In 2011, she found that Sca1 was more than just a biomarker -- it played a key role in creating and maintaining the stem-like quality in cancer cells.

She then looked to see if Sca1 works the same way in humans, and found a family of Ly6 genes that mapped to the same chromosomal location in humans where Sca1 resides in the mouse genome. The Ly6 family of genes was structurally similar to Sca1 as well.

This study was designed to determine if any of the genes in the Ly6 family are important in human cancer.

The researchers used 130 published, publicly available studies that included information on patients' genes and their cancer outcomes. Some studies were from the Georgetown Database of Cancer; others were available at the National Institutes of Health.

[1] http://www.eurekalert.org/pub_releases/2016-02/gumc-gft020416.php also see http://gumc.georgetown.edu/news/Gene-Family-Turns-Cancer-Cells-into-Aggressive-Stem-Cells-That-Keep-Growing

They discovered that four different members of the family -- Ly6D, Ly6E, Ly6H, or Ly6K -- are not active in normal tissue but are expressed in bladder, brain and central nervous system, colorectal, cervical, ovarian, lung, head and neck, pancreatic and prostate cancers. Investigators also found that high expression of these genes are linked to poor outcomes and reduced survival in ovarian, colorectal, gastric, lung, bladder and brain and central nervous system cancers.

"Correlation between Ly6 gene expression and poor patient survival in multiple cancer types indicate that this family of genes will be important in clinical practice -- not only as a marker of poor prognosis, but as targets for new drugs," Upadhyay says.

This study of big data supports the "cancer moonshot" proposal to speed up research announced by President Obama at this year's State of the Union address, Upaddhyay says. "The cancer field makes rapid progress when researchers share data and this study, which examines the work of scores of research teams, illustrates what can be done."

"We applied bioinformatic tools to explore the clinical significance of increased LY6 in survival outcome in multiple cancer types. Systems biology tools are critical for steering basic research to solve critical clinical challenges and identify novel signaling nodes such as this one," says co-author Subha Madhavan, PhD, director of the Innovation Center for Biomedical Informatics at Georgetown.

The LY6 human genes have been studied in relationship

2 LY6 GENES

From the paper by Luo, L., et al which we commenced this discussion with we have the following summary and detail:

The role of human Ly6 gene family is only beginning to be appreciated in recent literature. To study the significance of Ly6 gene family members, we have visualized one hundred thirty gene expression omnibus (GEO) dataset using Oncomine (Invitrogen) and Georgetown Database of Cancer (G-DOC). This analysis showed that four different members Ly6D, Ly6E, Ly6H or Ly6K have increased gene expressed in bladder, brain and CNS, breast, colorectal, cervical, ovarian, lung, head and neck, pancreatic and prostate cancer than their normal counterpart tissues. Increased expression of Ly6D, Ly6E, Ly6H or Ly6K was observed in sub-set of cancer type.

The increased expression of Ly6D, Ly6E, Ly6H and Ly6K was found to be associated with poor outcome in ovarian, colorectal, gastric, breast, lung, bladder or brain and CNS as observed by KM plotter and PROGgeneV2 platform.

The remarkable findings of increased expression of Ly6 family members and its positive correlation with poor outcome on patient survival in multiple cancer type indicate that Ly6 family members Ly6D, Ly6E, Ly6K and Ly6H will be an important targets in clinical practice as marker of poor prognosis and for developing novel therapeutics in multiple cancer type.

The lymphocyte antigen-6 (Ly6) complex, a group of alloantigens, was first discovered in mice approximately 40 years ago on lymphocytes. Ly6 family members are evolutionary conserved and have been mapped to human chromosome 8, in particular, the 8q24.3 locus, which is syntenic to murine chromosome 15.

The founding Ly6 member CD59 was identified in human lymphoid cells with a role in the complement membrane attack complex and T cell activation. To date, 20 human Ly6 proteins, ranging from 11-36 kDa, have been identified and categorized as either transmembrane or secretory based on the availability of a GPI-anchored signal sequence.

Ly6 family is located on chromosome 8q24 alongside c-Myc. The somatic copy number gain in 8q has been associated with most prevalent copy number gain in multiple cancer types. Ly6E and Ly6K have been implicated in development of novel therapeutics in multiple cancers. We have previously shown that increased levels of Ly6A/E (Sca-1) promote breast tumorigenesis via disruption of TGF-β signaling and suppression of GDF10 expression in mouse models. GDF10 has been shown to regulate epithelial to mesenchymal transition, growth and invasion in oral squamous cell carcinoma.

These finding suggest that Ly6 genes family members have important role multiple cancer but a comprehensive analysis of multiple members of Ly6 gene family and its relation to cancer patient survival is lacking.[2]

[2] http://www.ncbi.nlm.nih.gov/gene/57152

This presents a strong argument for broad prognostic value. However the specific reasons for its prognostic value is still a bit tenuous. We shall examine the paper in some further detail while also examining other prior research. The intrigue here is the nexus with immune response mechanisms.

2.1 SUMMARY

LY6 are closely associated with a variety of immune responses. We provide a brief summary from Kong et al who state:

1. LY-6D *is expressed exclusively on normal squamous epithelia and transitional epithelium and their malignant counterparts. LY-6D is an effective target antigen for diagnosis and therapeutic exploitation in the management of patients with head and neck squamous cell carcinoma (HNSCC) and micrometastases to lymph nodes from HNSCC.*

The impact of the LY-6D has some correlative effect. The HNSCC relationship may be causative or surreptitious. This specific RNA is linked to blocking infections and causing structural organization changes. However as with all the specific details of these processes is uncertain.

2. LY-6E *(RIG-E, stem cell antigen 2, and thymic shared antigen) are expressed in squamous cells, peripheral blood, and bone marrow cells. LY-6E plays an important role in T-cell differentiation, activation of the T-cell receptor signaling pathway, and proliferation and differentiation of T2ECs (transforming growth factor [TGF]-α and TGF-β induced erythrocytic cells) that self-renew primary avian erythroid progenitors. Interestingly, LY-6E is highly expressed in various human cancers, such as colon cancer and malignant kidney cancer. Thus, LY-6E affects tumorigenesis by changing cell proliferation and differentiation.*

The impact on proliferation and differentiation is a key factor. Just what the process is does not seem to be well understood. Furthermore, as with the rest of these RNAs there is the causal factor as well.

3. LY-6H *is highly expressed in the human brain, such as the cerebral cortex and also in acute lymphoblastic leukemia cells; thus, LY-6H might play an important role in both the central nervous (CNS) and immune systems.*

There is with LY6H a series of speculative relationships as well. This specific one seems the least understood amongst the group.

4. LY-6K*, as a cancer-testis antigen, was initially identified as a HNSCC diagnostic and therapeutic target antigen similar to LY-6D. Elevated LY-6K is a serologic diagnostic biomarker and therapeutic target for breast cancer, lung and esophageal carcinomas, bladder cancer, and esophageal squamous cell carcinoma (ESCC). Induced LY-6K regulates cell growth, invasion, and migration of lung, esophageal, breast, and bladder cancer cell lines. Additionally, LY-6K is an immunotherapeutic target of cancer vaccine therapies because LY-6K stimulates cytotoxic T lymphocytes that have specific cytotoxic activity against ESCC cells that endogenously express LY-6K. Taken together, these results suggest that inhibiting LY-6K expression may be a*

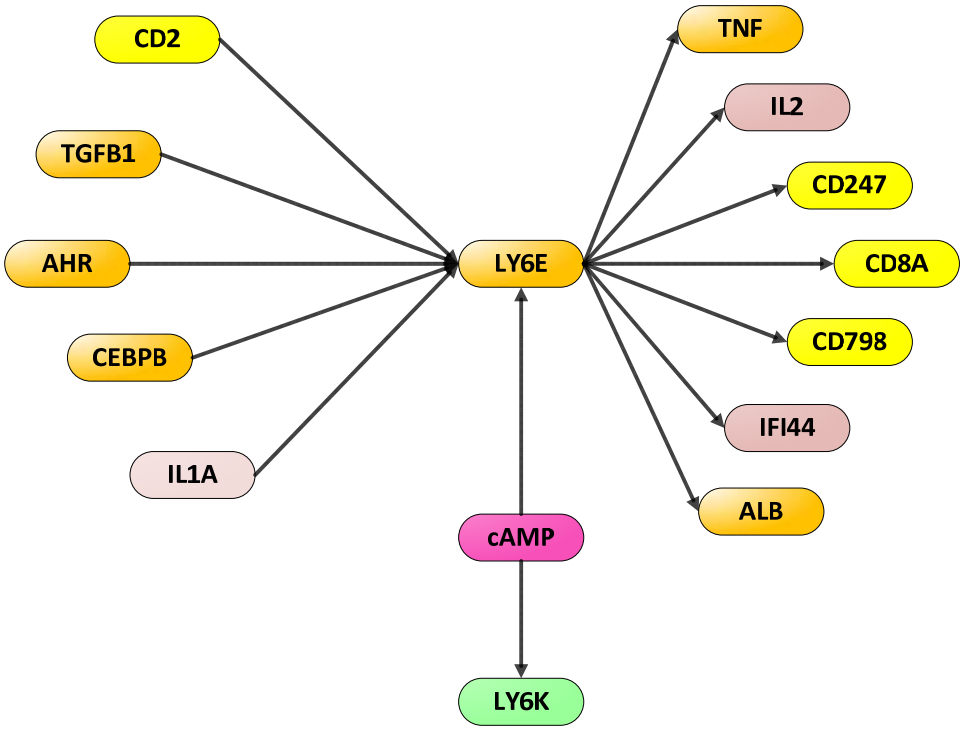
promising target for control of these cancers and that targeting LY-6K may be a novel cancer therapy strategy.

The therapeutic targeting of LY-6K has interest as expressed above but the adventitious presence may be neither causative nor reflective of a process whose control can inhibit the cancer. Frankly this seems to be a general problem with this class of RNAs.

We will now examine each of these in some minor detail.

2.2 LY6E

From NCBI[3] we have LY6E at 8q24.3. As shown in the target:



Also as Feng et al note in a prior study on SLE, an immune related disease:

The identification of biomarkers helps to perform early diagnosis, thus benefits the outcome of patients with systemic lupus erythematosus (SLE), in which delayed treatment has been proposed as an independent adverse prognostic factor.

In this study, we assessed the values of expression levels of five type I interferon (IFN)-inducible genes (LY6E, OAS1, OASL, MX1, and ISG15) and total IFN score for the diagnosis of SLE. Quantitative real-time PCR was applied to determine gene expressions at transcription level in

[3] http://www.ncbi.nlm.nih.gov/gene/4061 LY6E lymphocyte antigen 6 complex, locus E [Homo sapiens (human)]

peripheral blood from 69 SLE patients, 42 patients with other connective tissue diseases, and 26 normal controls.

Expressions of five genes and IFN score, calculated according to the expressions of IFN-inducible genes, were all significantly increased in SLE patients compared to those in normal subjects and disease controls. IFN score was not related to age, gender, and the dose of steroids, but weakly correlated with SLE disease activity index.

None of the gene expression was associated with concomitant infection status or elevated antibodies against Epstein-Barr (EB) virus in SLE. Both modified IFN score (calculated by the expression of three major IFN-inducible genes) and LY6E level showed good diagnostic accuracy in discriminating between SLE patients and disease controls as well as normal subjects (area under the receiver operating characteristic curve was 0.812 and 0.815, respectively), with 70-80 % specificity and 70-80 % sensitivity at the cutoff of 2.37 and 3.23. In conclusion, high IFN-inducible gene expression is constitutional for SLE patients.

The modified IFN score or the LY6E level alone may serve as good biomarkers for SLE diagnosis.

From Xu et al we have further evidence of the immune elements of LY6:

Owing to ongoing recognition of pathogen-associated molecular patterns, immune activation and upregulation of IFN-stimulated genes (ISGs) are sustained in the chronically infected host. Albeit most ISGs are important effectors for containing viral replication, some might exert compensatory immune suppression to limit pathological dysfunctions, although the mechanisms are not fully understood.

In this study, we report that the ISG lymphocyte Ag 6 complex, locus E (LY6E) is a negative immune regulator of monocytes. LY6E in monocytes negatively modulated CD14 expression and subsequently dampened the responsiveness to LPS stimulation in vitro. In the setting of chronic HIV infection, the upregulation of LY6E was correlated with reduced CD14 level on monocytes; however, the immunosuppressive effect of LY6E was not adequate to remedy the hyperresponsiveness of activated monocytes.

Taken together, the regulatory LY6E pathway in monocytes represents one of negative feedback mechanisms that counterbalance monocyte activation, which might be caused by LPS translocation through the compromised gastrointestinal tract during persistent HIV-1 infection and may serve as a potential target for immune intervention.

They conclude:

Our data revealed that LY6E exerts its role through downregulating CD14 expression at the transcriptional step but not the stage of protein stability or degradation. The possibility remains that the transcriptional machinery involved in CD14 expression, such as the activity of transcription factors and the transcriptional status of the promoter region, may be influenced by the LY6E pathway. Another avenue for further investigation is how the LY6E pathway is

triggered. To date, no LY6E ligand has been identified. It would also be interesting to uncover how LY6E, as a GPI-anchored protein, introduces an intracellular signaling cascade. Further investigations will be pursued to address these questions.

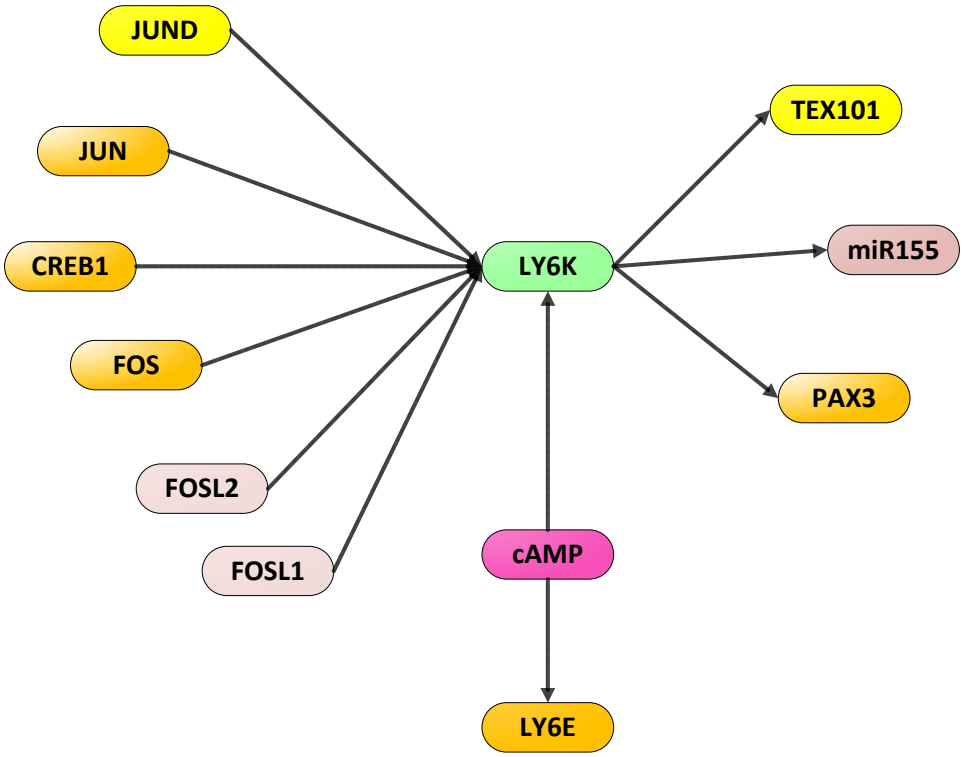
The following is a summary from NCBI of some of the key genes regulated or interacting with this RNA.

Gene	***Description***
CAMP	This gene encodes a member of an antimicrobial peptide family, characterized by a highly conserved N-terminal signal peptide containing a cathelin domain and a structurally variable cationic antimicrobial peptide, which is produced by extracellular proteolysis from the C-terminus. In addition to its **antibacterial, antifungal, and antiviral** activities, the encoded protein functions in cell chemotaxis, **immune mediator induction, and inflammatory response regulation**.
CD2	CD2 is a surface antigen of the human T-lymphocyte lineage that is expressed on all peripheral blood T cells. It is one of the earliest **T-cell markers**, being present on more than 95% of thymocytes; it is also found on some natural killer cells but not on B lymphocytes. Monoclonal antibodies directed against CD2 inhibit the formation of rosettes with sheep erythrocytes, indicating that CD2 is the erythrocyte receptor or is closely associated with it
TNF	This gene encodes a **multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor** (TNF) superfamily. This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuroprotective function of this cytokine.
IL2	The protein encoded by this gene is a **secreted cytokine that is important for the proliferation of T and B lymphocytes**. The receptor of this cytokine is a heterotrimeric protein complex whose gamma chain is also shared by interleukin 4 (IL4) and interleukin 7 (IL7). The expression of this gene in mature thymocytes is monoallelic, which represents an unusual regulatory mode for controlling the precise expression of a single gene. The targeted disruption of a similar gene in mice leads to ulcerative colitis-like disease, which suggests an essential role of this gene in the immune response to antigenic stimuli. [
ALB	Albumin is a soluble, monomeric protein which comprises about one-half of the blood serum protein. Albumin functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones and plays a role in stabilizing extracellular fluid volume. Albumin is a globular unglycosylated serum protein of molecular weight 65,000. Albumin is synthesized in the liver as preproalbumin which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin.
TGFB1	This gene encodes a member of the t**ransforming growth factor beta (TGFB) family of cytokines**, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types. Many cells have TGFB receptors, and the protein positively and negatively regulates many other growth factors. The secreted protein is cleaved into a latency-associated peptide (LAP) and a mature TGFB1 peptide, and is found in either a latent form composed of a TGFB1 homodimer, a LAP homodimer, and a latent TGFB1-binding protein, or in an active form composed of a TGFB1 homodimer. The mature peptide may also form heterodimers with other TGFB family members. This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease.
CD8A	The CD8 antigen is a cell surface glycoprotein **found on most cytotoxic T lymphocytes that mediates efficient cell-cell interactions within the immune system**. The CD8 antigen acts as a coreceptor with the T-cell receptor on the T lymphocyte to recognize antigens displayed by an antigen presenting cell in the context of class I MHC molecules. The coreceptor functions as either a homodimer composed of two alpha chains or as a heterodimer composed of one alpha and one beta chain. Both alpha and beta chains share significant homology to immunoglobulin variable light

Gene	*Description*
	chains. This gene encodes the CD8 alpha chain. Multiple transcript variants encoding different isoforms have been found for this gene.
CD247	The protein encoded by this gene is **T-cell receptor** zeta, which together with T-cell receptor alpha/beta and gamma/delta heterodimers, and with CD3-gamma, -delta and -epsilon, forms the T-cell receptor-CD3 complex. The zeta chain plays an important role in coupling antigen recognition to several intracellular signal-transduction pathways. Low expression of the antigen results in impaired immune response. Two alternatively spliced transcript variants encoding distinct isoforms have been found for this gene

2.3 LY6K

The putative pathways for LY6K is describes below.



Matsuda et al note as to LY6K:

The LY6 family members are assumed to have functions related to cell signalling and/or cell adhesion although the precise role of LY6K in carcinogenesis is still unknown. To gain further insight into which genes are affected by LY6K gene expression, we performed gene expression analysis of the LY6K transfectant.

The functional annotations of the upregulated genes after LY6K transfection were distributed among nine categories including cell cycle, transcription, and signal transduction. Several +clusters of genes in our profile are oncogenic molecules contributing to cancer development, for example, E2F genes, insulin-like growth factors, cell division cycle genes, a-/btubulines , and

zinc finger proteins. These results suggest that LY6K promotes and activates cell-cycle-related genes in BC.

Further investigation is necessary to test this hypothesis. In our cohort, there was no significant relationship between the LY6K mRNA expression and clinicopathological parameters.

Regional epigenetic silencing and activation of multiple genes unrelated to chromosomal alterations may affect the pathological parameters of individual tumours. More precise studies with various experiments are needed to explain these phenomena.

From NCBI LY6K is located at 8q24.3[4]. From Kong and Park:

The human LY6K (lymphocyte antigen 6 complex locus K) belongs to the Ly-6/urokinase-type plasminogen activator receptor (uPAR)2 superfamily, which can be divided into two subfamilies based on the glycosylphosphatidylinositol-anchoring signal sequence: one is the glycosylphosphatidylinositol anchored transmembrane proteins, which include the retinoic acid-induced gene E (human LY6E), the E48 antigen (human LY6D), LY6H, prostate stem cell antigen, CD59, or protectin, lynxl, and uPAR (1–3).

The other is a secretory protein without a glycosylphosphatidylinositol-anchoring signal sequence, which includes SLURP-1 and SLURP-2 . The glycosylphosphatidylinositol- anchored uPAR modulates tumor invasion, growth, and metastasis via the integrin-related Ras/ERK signaling pathway (5, 6). The LY6K was first identified as a molecular marker for head-and-neck squamous cell carcinoma .

Previous studies have shown that cancer-testis antigen LY6K could be a diagnostic biomarker and a therapeutic target for breast cancer, nonsmall cell lung carcinoma, bladder cancer, and esophageal squamous cell carcinoma (8–11).

In breast cancer, elevated LY6K induces cell invasion and metastasis by activating the Raf-1/MEK/ERK signaling pathway and up-regulating expression of matrix metalloproteinase proteins MMP-2 and MMP-9 . Likewise, LY6K regulates cell growth, migration, and invasion in bladder cancer cell lines . However, the molecular mechanisms that mediate regulation of LY6K gene expression in cancer are unknown.

Again from Kong and Park:

LY6K is a cancer biomarker and a therapeutic target that induces invasion and metastasis. However, the molecular mechanisms that determine human LY6K transcription are completely unknown. To elucidate the mechanisms involved in human LY6K gene regulation and expression, multiple cis-elements were predicted using TRANSFAC software, and the LY6K regulatory region was identified using the luciferase assay in the human LY6K gene promoter.

[4] http://www.ncbi.nlm.nih.gov/gene/54742 LY6K lymphocyte antigen 6 complex, locus K [Homo sapiens (human)]

We performed ChIP, EMSA, and supershift assays to investigate the transcription factor activity on the LY6K promoter, and the effect of a SNP and CpG site methylation on AP-1 transcription factor binding affinity. AP-1 and the CREB transcription factor bound to LY6K promoter within -550/-1, which was essential for LY6K expression, but only the AP-1 heterodimer, JunD, and Fra-1, modulates LY6K gene transcriptional level.

A decrease in LY6K was associated with the SNP242 C allele, a polymorphic G/C-SNP at the 242 nucleotide in the LY6K promoter region (rs2585175), or methylation of the CpG site, which was closely located with the AP-1 site by interfering with binding of the AP-1 transcription factor to the LY6K promoter. Our findings reveal an important role for AP-1 activation in promoting LY6K gene expression that regulates cell mobility of breast cancer cells, whereas the SNP242 C allele or methylation of the CpG site may reduce the risk of invasion or metastasis by interfering AP-1 activation.

As Matsuda et al note:

In summary, our studies firstly demonstrated that LY6K gene may have an oncogenic activity in human BC (Bladder Cancer) and chromosomal gain locus of 8q24.3 where LY6K gene harbours may have a critical role for BC development.

We conducted experiments to clarify the gain and loss of functions using a stable LY6K-transfected BC cell line and found that LY6K might have an oncogenic function, suggesting that it is a promising candidate for molecular targeting of human BC.

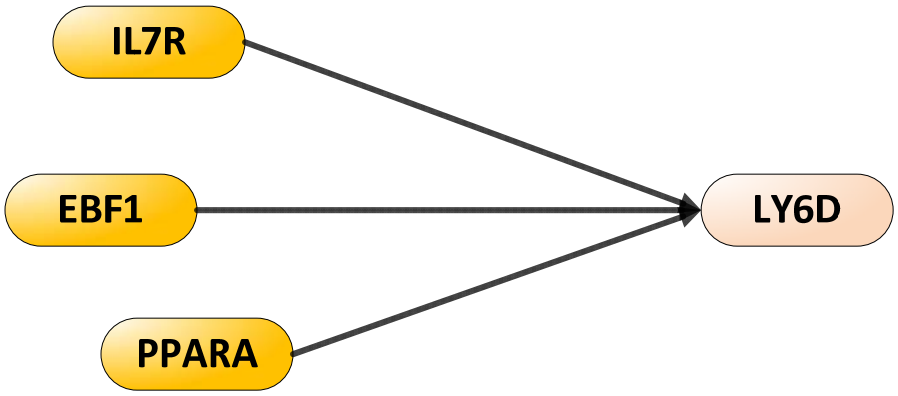
In a similar fashion we present a summary from NCBI of the related genes:

Gene	***Description***
JUND	The protein encoded by this intronless gene is a member of the JUN family, and a functional component of the AP1 transcription factor complex. This protein has been proposed to protect cells from p53-dependent senescence and apoptosis. Alternative translation initiation site usage results in the production of different isoforms
JUN	This gene is the putative transforming gene of avian sarcoma virus 17. It encodes a protein which is highly similar to the viral protein, and which interacts directly with specific target DNA sequences to regulate gene expression. This gene is intronless and is mapped to 1p32-p31, a chromosomal region involved in both translocations and deletions in human malignancies
CREB1	This gene encodes a transcription factor that is a member of the leucine zipper family of DNA binding proteins. This protein binds as a homodimer to the cAMP-responsive element, an octameric palindrome. The protein is phosphorylated by several protein kinases, and induces transcription of genes in response to hormonal stimulation of the cAMP pathway. Alternate splicing of this gene results in two transcript variants encoding different isoforms
FOS	The Fos gene family consists of 4 members: FOS, FOSB, FOSL1, and FOSL2. These genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1. As such, the FOS proteins have been implicated as regulators of cell proliferation, differentiation, and transformation. In some cases, expression of the FOS gene has also been associated with apoptotic cell death.

Gene	*Description*
FOSL2	The Fos gene family consists of 4 members: FOS, FOSB, FOSL1, and FOSL2. These genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1. As such, the FOS proteins have been implicated as regulators of cell proliferation, differentiation, and transformation.
FOSL1	The Fos gene family consists of 4 members: FOS, FOSB, FOSL1, and FOSL2. These genes encode leucine zipper proteins that can dimerize with proteins of the JUN family, thereby forming the transcription factor complex AP-1. As such, the FOS proteins have been implicated as regulators of cell proliferation, differentiation, and transformation. Several transcript variants encoding different isoforms have been found for this gene.
PAX3	This gene is a member of the paired box (PAX) family of transcription factors. Members of the PAX family typically contain a paired box domain and a paired-type homeodomain. These genes play critical roles during fetal development. Mutations in paired box gene 3 are associated with Waardenburg syndrome, craniofacial-deafness-hand syndrome, and alveolar rhabdomyosarcoma. The translocation t(2;13)(q35;q14), which represents a fusion between PAX3 and the forkhead gene, is a frequent finding in alveolar rhabdomyosarcoma. Alternative splicing results in transcripts encoding isoforms with different C-termini

2.4 LY6D

The LY6D gene is located at 8q24[5].



Note that this is a bit from the other two previous locations. From Kurosawa et al we have:

In order to identify membrane proteins whose expression is induced by X-ray irradiation, we developed an antibody (Ab)-directed strategy using a phage Ab library. X-Ray-irradiated cells were screened with a phage Ab library in the presence of a large excess of polyclonal Abs prepared against membrane proteins that are commonly present at the surface of both X-ray-irradiated and nonirradiated cells. After isolation of Ab that bound only to X-ray-irradiated cells, the antigen was identified using MS. Using this approach, we found that expression of LY6D is induced in MCF10A cells by X-ray irradiation. The induction of LY6D expression is

[5] http://www.ncbi.nlm.nih.gov/gene/8581 LY6D lymphocyte antigen 6 complex, locus D [Homo sapiens (human)]

triggered through a pathway regulated by ATM, CHK2 and p53. This method is a new Ab-directed proteomic strategy for analysis of membrane proteins, and is applicable to various biological phenomena in situations in which both target molecule-expressing cells and nonexpressing cells are available.

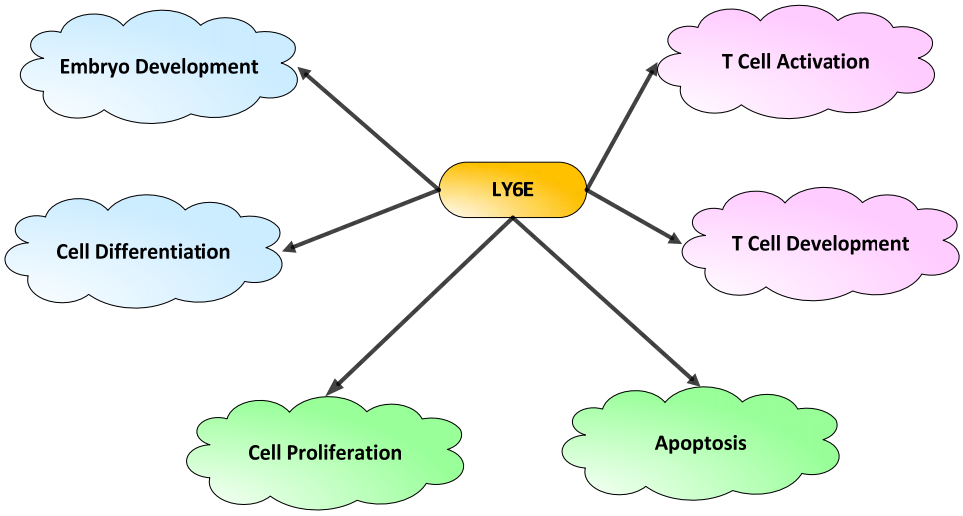
2.5 LY6H

From NCBI we have LY6H at 8q24.3[6]. From Horie at al:

The Ly6 family of genes encodes glycosylphosphatidylinositol-anchored cell surface glycoproteins expressed on various types of cells. Intriguing patterns of expression of Ly6 genes on specific subpopulations of lymphoid and myeloid cells suggest that Ly6 molecules may be involved in the development and homeostasis of hematopoietic cells. We have isolated a new member of the human Ly6 gene family, LY6H, from a human fetal brain cDNA library. Fluorescence in situ hybridization and radiation hybrid analyses assigned LY6H to chromosome 8, where other members of the Ly6 gene family are also located. Northern analysis revealed that LY6H is highly expressed in particular subdivisions of human brain and also in MOLT-3 and -4 acute lymphoblastic leukemia cells. These data suggest that LY6H may play a role(s) in both the central nervous system and the immune system

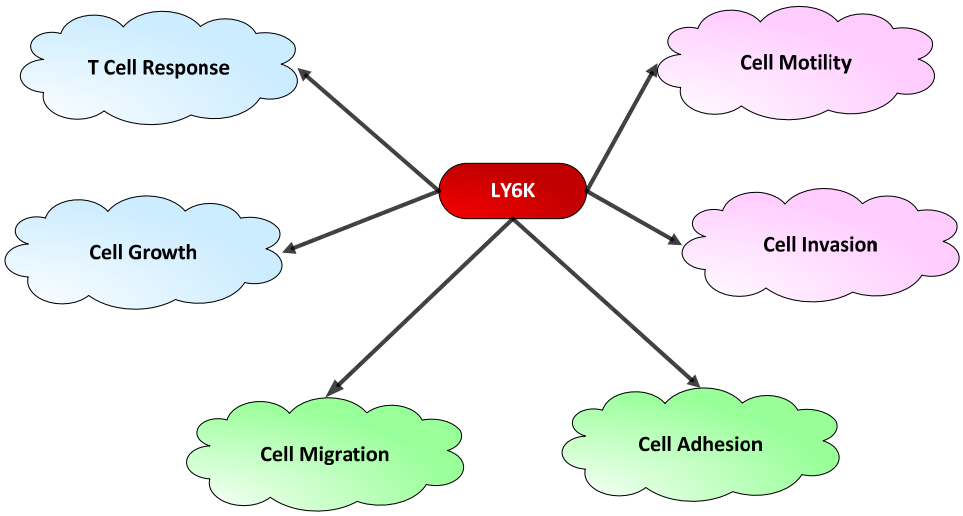
2.6 SOME EFFECTS

The following are some general effects resulting from the LY6 expression.



And

[6] http://www.ncbi.nlm.nih.gov/gene/4062 LY6H lymphocyte antigen 6 complex, locus H [Homo sapiens (human)]


T Cell Response
Cell Motility
LY6K
Cell Growth
Cell Invasion
Cell Migration
Cell Adhesion

3 OBSERVATIONS

Unlike many other markers these specific ones appear to be related to immune responses and may be reflective of an aggressive tumor state because of the response of the immune system in general trying to deal with these cells. The authors and the press release state:

…shows that this Ly6 family of genes allows cancer cells to act like cancer stem cells -- which keep dividing and growing without pause. "These are remarkable findings. We believe this family of genes produces cancer that easily metastasizes, is drug resistant and very difficult to destroy,"

The conclusion may be a bit more than what has been shown. The characteristics of stem cells are still in many ways a work in progress. We have examined some of these in the area of prostate cancer and there is yet to be a definitive description of the development and characterization of a stem cell in this case. As Stolpe states:

Despite remaining uncertainties and ongoing research it is possible to draw up a model for the role of (cancer) stem cells in both the initiation and progression of cancer towards metastasis. The cancer stem cell of origin and the cancer stem cell are, despite phenotypic similarities, genotypically different entities.

Given the right circumstances provided by a combination of genomic changes and biochemical and physical interactions with its microenvironment, an epithelial cancer cell may undergo a phenotypic epithelial mesenchymal transition (EMT) towards a cancer stem cell. This transition conveys upon the cell crucial stem cell-like abilities which facilitate migration into the blood circulation as an individual circulating tumor cell, survive there, and subsequently seed into organ tissue where, once more in close interaction with its microenvironment, the process of clonal self renewal may start, leading to a metastatic tumor.

Both in the primary tumor as well as in the metastatic tumor, partial differentiation of the cancer stem cell progeny leads to phenotypic heterogeneity. Throughout this complex process of cancer metastasis similarities with the way stem cells function during embryonic development, including the signaling pathways that mediate these functions, are evident. Deeper insight in the EMT process, plasticity of the resulting cancer stem cells, and the role of cancer stem cells in the metastatic process is expected to lead to novel anti-metastatic cancer therapies. Emerging human in vitro cancer models in the form of "organ-on-a-chip" may contribute valuable novel research tools to achieve this aim…..

In the concept described, cancer stem cells do not represent the stem cell of origin of the cancer, but originate from a cancer cell which lost its epithelial properties and instead newly acquired certain stem cell characteristics, enabling it to contribute to tissue invasion and metastasis. Combining experimental evidence from different research areas into a conceptual view on the role of cancer stem cells does not mean that the concept itself has been experimentally proven. Instead, it may provide useful guidance to future design of experiments aiming at elucidating the complex ways in which a cancer metastasizes. Obtaining such in depth knowledge is key to

developing more effective drugs to tackle metastatic behavior of cancer – since metastasis causes death.

Furthermore as Ni et al state:

Cancer stem cells (CSCs), a minority population of cancer cells characterised by self-renewal and tumor initiation, have gained intense attention as they not only play a crucial role in cancer recurrence but also contribute substantially to chemoresistance. As such, a number of mechanisms in chemoresistance have been identified to be associated with CSCs. Therefore, a thorough and integral understanding of these mechanisms can identify novel biomarkers and develop innovative therapeutic strategies for CaP treatment. Our recent data have demonstrated CSCs are associated with CaP chemosensitivity. In this review, we discuss the roles of putative CSC markers in CaP chemoresistance and elucidate several CSC-associated signaling pathways such as PI3K/Akt/mTOR, Wnt/β-catenin and Notch pathways in the regulation of CaP chemoresistance. … Despite the debate on CSCs' existence, cancer is becoming more recognized as a heterogenenous disease with hierarchies of subpopulations that demonstrate a variety of phenotypes. Two models have been proposed to explain tumor heterogeneity: the stochastic and hierarchical models (Fig. 1). While the stochastic model proposed that all cells within a tumor are biologically homogenous and therefore have equal capacity to regenerate the tumor, the hierarchical model (also referred to as the CSC model) suggested that only a small subset of tumor cells possesses the capacity to regenerate the tumor [10, 15]. According to the hierarchical model, it should be possible to separate tumor cells into subpopulations that are tumor initiating and non-tumor initiating. The tumor-initiating cells, also referred to as CSCs, are defined by their capacity for self-renewal, potential to differentiate into any cells in a tumor, and proliferative capacity to drive expansion of the tumor.

Namely the CSC is still an uncertain entity and thus one could question the strong statement made by the authors regarding the putative use of these LY6 markers.

And also as Massague and Obenauf state:

Adult stem cells reside in specialized niches that provide cues that help to maintain a balance between stem-cell proliferation and quiescence as well as self-renewal and differentiation. Stem-cell niches are rich in developmental and self-renewal signals, such as hedgehog, Wnt, members of the TGF-β family and the chemokine CXCL12. Tumours are thought to arise from mutant stem cells in their native niches or from the progeny of cells that retain their tumour-initiating capacity and benefit from these niche signals. After cancer stem cells disperse to distant sites, their survival and tumour-initiating potential can benefit similarly from interactions with specialized niches. Evidence suggests that prostate-carcinoma stem cells occupy native haematopoietic stem-cell niches in the bone marrow. The CXCL12 receptor CXCR4 is a marker and mediator of breast-cancer metastasis to CXCL12-rich bone-marrow sites71. Breast tumours that are rich in a CXCL12-secreting mesenchymal stroma select for CXCL12- responsive cancer-cell populations that are predisposed to survive in the bone marrow.

Thus the argument for these being stem cell markers has some problematic issues.

The final conclusions made in the Press Release are:

They discovered that four different members of the family -- Ly6D, Ly6E, Ly6H, or Ly6K -- are not active in normal tissue but are expressed in bladder, brain and central nervous system, colorectal, cervical, ovarian, lung, head and neck, pancreatic and prostate cancers. Investigators also found that high expression of these genes are linked to poor outcomes and reduced survival in ovarian, colorectal, gastric, lung, bladder and brain and central nervous system cancers.

As we have noted these four LY6 RNAs are highly related to immune system responses but are not shown to be either causative or related in some chain to specific causative genetic changes. We would argue that there may be a bit of stretching both on the stem cell issue as well as the issues related to involvement.

Finally there does not appear to be any therapeutic targets presented here as well.

Overall this is an interesting addition to the literature but it will require in my opinion some further substantiation of its overall merit as a prognostic tool.

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