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Biomarkers – The Search for the Missing Link

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The biggest leap in the age of bio-informatics has been the advent of next generation sequencing. Its latest discovery, the presence of non-coding RNA (ncRNA), was one of the least explored domains of human genome in the start of the century. Though it is believed to constitute more than 50–70% of the human genome, without being able to assess its potential role in human evolution; it was considered the dark matter by some and junk DNA by others [1].

Though evident way back much earlier, that the divergent protein sequences required for the so-called chimp to human evolution could not be explained by the highly conserved protein sequences [2]. Britten and Davidson in 1971 were the first to perpetuate the idea that RNA might interact with genomic DNA leading to the specific molecular profile of each cell [3]. But the idea remained neglected for next two decades until Brannan et al., finally proved the existence of genomic activities of non-coding RNA's [4].

The non-coding RNA is divided into 2 groups:

1. Small non-coding RNA's
2. Long non-coding RNA's

The small ncRNA includes the much studied micro RNAs (miRNAs); that has found way in every textbook that deals with genetic basis of diseases; others being small interfering RNAs, PIWI interacting RNAs and some bacterial regulating RNAs [5].

The long ncRNA (lncRNA) are 200 nucleotide long mRNA likes transcripts with an open reading frame. A unique character shared by all long noncoding RNA is that most of these are transcribed by RNA polymerase II and is polyadenylated. Though dismissed earlier as technological glitch, we are now beginning to understand, that they may be key to cellular differentiation, development and metabolism and may be the seat of human evolution [6].

The only study that has proved its complexity other than that of human genetics is of cancer cell biology. Our understanding of cancer cell biology is limited due to our inability to define the natural history of most of these diseases. The addition of lncRNA in the list of deregulated genetic material makes it even more complex. The study of cancer biomarkers currently have been directed on the prognostication of the patient and very rarely diagnosis of the patient. The major cause of this has been the protein first approach; use of proteins as biomarkers has a big fallacy inherent to the fact that they are surrogates to the actual process that is going on in a malignant cell. With the ability to analyse lncRNA directly we might be able to detect at a very early stage of the disease and also help in understanding the missing links in the current understanding of tumorigenesis. LncRNA have been found to be elevated in various carcinomas and there relative over-expression and under expression can be matched with controls which can then be used for better prognostication of the disease [7].

A few lncRNAs like PCA3 have been found in patient urine samples that have shown to be more sensitive and specific that PSA for diagnosing prostate cancer. An ideal biomarker is one that can be easily obtained from the patient in the least possible invasive manner [8]. Similar to PCA3, another lncRNA HULC could be detected easily in blood samples from patients suffering from HCC and was found have direct correlation with the metastatic potential of the disease process [9]. HOTAIR transcript another lncRNAs has been noted to be deregulated in cells which have increased ability to metastasize and in tumour with poor prognosis esp. in breast carcinoma model, in another study it has been shown to be an important marker to suggest recurrence of HCC after hepatic transplantation [10]. The ability of the lncRNA to remain stable in the context of their extracellular presence is a matter of scrutiny and new studies are being conducted which will await the arrival of novel lncRNA that can be detected by economically sound and commercially viable options.

Finally the ability to silence these lncRNAs and its effects in in-

vitro models are worth investigating. Use of RNAi-mediated gene silencing as a novel treatment modality could serve as the next frontier in the management of these diseases. In a study on PCNCR1 (Prostrate cancer Non coding RNA 1) it was noted that by achieving its knockdown in tumour cells, they could inhibit the Prostrate Cancer cells from multiplying and also inhibition the trans-activating ability of these cells [11].

Lnc-RNA have been classified in view of their proximity to genetic components as:

- Sense
- Antisense
- Bidirectional
- Intronic
- Intergenic

Epigenetics, nuclear imports, alternative splicing, small RNA precursors, mRNA decay and structural components are few of roles that these lncRNAs are already documented to perform in the human cellular machinery [12]. Though our library is limited to a few 200 of these lncRNAs, accumulating reports of mutations in the lncRNA suggest that they might play an important role in cancer cell biology.

It would be worthwhile to review few of our achievements with respect to this entity within the last decade

Genes that are linked to tumorigenesis can be classified as

- Tumour suppressor genes
- Oncogenes

Tumour suppressor genes are considered to be the protectors of human genome and they are known to safeguard the organism from deleterious mutations and any other somatic or germ line transformations. On the other hand genes that are known to activate or facilitate tumorigenesis are known as oncogenes. The next generation sequencing models have allowed the practitioners to identify and demonstrate the role of lncRNA as both tumour suppressors and oncogenes.

Oncogenic lncRNAs

HOTAIR–HOX transcript antisense intergenic RNA

HOTAIR is a 2200 base gene located in HOXC locus on chromosome 12q13.13 and was initially discovered as the repressor of HOXD gene. The HOX locus however is known to produce a variety of lncRNA which may suggest HOTAIR may be one of many regulatory RNA in this region. In a landmark study by Yao et al., it was noted that HOTAIR was expressed more often in primary and metastatic human cancers, with over 2000 fold increase in transcription from the control tissue [13]. Further it was noted that in patients with primary breast carcinoma with increased HOTAIR copies had poorer outcomes in terms of overall survival outcome and metastatic potential. In accordance to Koch postulates when these RNA were introduced in mouse mammary fat pads an increase in the tumour duplication rate was observed while knockout models showed decreased invasiveness of the tumour cells.

A major shift in understanding of how HOTAIR acts as an oncogene has come about by the knowledge of PRC2 complexes. The Polycomb Repressive Complex 2 (PRC2) is a group of proteins including H3K27 methylase, EZH2, SUZ12, and EED. These complexes are known to cause epigenetic changes in the mammalian genetic profile regulating pluripotency and tumorigenesis. Another set of such proteins are the LSD1/coREST complex which also is one of the targets of HOTAIR that leads to repression of various activating transcripts. The PRC2 is known to bind to the 5' region of HOTAIR, and LSD1 to 3' which then leads to histone methylation and demethylation respectively. The ying-yang nature of HOTAIR mediated methylation and demethylation of leads to generation of gain of repressive histone marks and loss of activating histone marks causing overall silencing of the genetic template it acts on [14]. Though the exact mechanism is yet to be elucidated, it has been proposed that HOTAIR might also be responsible for epithelial to mesenchymal transformation that is responsible for increased cancer cell invasiveness through the PRC2 complex discussed above.

ANRIL–Antisense ncRNA in the INK4 locus

The fabled dark matter of the human genome ncRNA is known to contain antisense transcripts which have the capacity to alter the sense genes. Some of these protein encoding genes are known to produce tumour suppressor genes like p15INK4A, p14ARF, p16INK4A, and activation of antisense RNA transcripts can lead to silencing of these genes which has been the basis of many tumour models [15]. ANRIL is one of such gene that is known to epigenetically silence the INK4A-ARF-INK4B locus by heterochromatin formation. With the activation of PRC1 and PRC2 complexes ANRIL is known to initiate repressive epigenetic marks on the above mentioned TSG locus and p15/CDKN2B which serves as the major driving force of this ncRNA-GENE-Tumour model [16].

MALAT1–Metastasis associated ling adenocarcinoma sequence transcript 1

MALAT1 with its locus at chromosome 11q13.1 is known to create alternative splicing sequences by phosphorylation of SR splicing factor (serine/arginine) and has chromosomal translocation breakpoints that are associated with malignant cell biology. Increased expression is noted in various cancers by altering the mobility of the tumour cells. It was first noted to be associated with poor prognosis in cases of primary non-small cell lung cancer tumours [17]. Predominantly residing in the nuclear speckles, this lncRNA is found in all normal tissues, but is specifically overexpressed in breast, pancreas, hepatocellular, colon, and prostate cancers.

The in-vitro studies to silence MALAT1 gene has been noted to reduce the invasiveness and mobility of tumour cells especially in lung adenocarcinoma model and cervical cancer models. Inhibition of MALAT1 activity leads to change in the splicing

sequence of pre-mRNA that is known to cause the above mentioned changes.

H19

H19 is a 2.3 kb lncRNA known for genomic imprinting and is only expressed from the maternal allele, and has important role in growth and development of the tissues. H19 has been found to be overexpressed in adult stem cell activation and in tissues undergoing malignant transformation. High expression is noted in various carcinomas originating from the breast, urinary bladder, oesophagus and hepatic metastasis which is associated with loss of imprinting at its locus [18-21]. The c-Myc gene has been associated with the activation of the H19 ncRNA with is considered to be the pivotal point in this model. Furthermore H19 receives a special mention because it serves as a transcriptional precursor to miR-675, a micro RNA that has a definite role in tissue development and differentiation [22]. Being processed from the first exon of H19 it is known to cause inhibition of Retinoblastoma gene locus.

Tumour Suppressor lncRNAs

LincRNA-p21

P53 the guardian of the genome is also known to induce many lncRNA to mediate its epigenetic actions and bring about its tumour suppressor effects. One of these regulatory lncRNA is a 3.1 kb transcript located proximally to Cdkn1a, a cell cycle regulator termed as lincRNA-p21 [23]. Deregulation of this transcript has been shown to cause mislocatisation of heterogeneous nuclear ribonucleoprotien k (hnRNP-K). Though further studies have not been able to directly elucidate the role of this lncRNA in tumorigenesis, this shows that lncRNA could well be a marker for p53 dysfunction.

GAS5–Growth arrest-specific 5

This is the first lncRNA to have shown to respond to external stimuli in form of response to glucocorticoids. This lncRNA is noted to be overexpressed in cells that have arrested growth and it further directs the cell towards apoptosis in starvation [24]. These transcripts act as molecular decoys and bind to the glucocorticoids nuclear receptor at the level of DNA, leading to reduction in cellular metabolism. Defects in this transcript could explain the survival of malignant cells in a nutrient scarce environment without undergoing apoptosis, as noted in few studies on a breast cancer model.

CCND1

This gene present in the promoter region of cyclin-D1 is known to act by inhibiting histone acetyltransferase such as CREB-binding protein and p300, by allosteric activation of TLS (Translocated in liposarcoma) gene, a sensor of DNA damage, resulting in down regulation of cyclin-D1. Inactivation of this gene causes unprecedented response of cyclin D1 causing uncontrolled rise in tumour clones [25].

This list is neither complete nor does it explore the limits of this new entity, but it gives us an insight to where we stand today in the world of biomarkers. With the study of lncRNAs gaining momentum especially with research projects like TCGA, a collaboration between the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI), that aims to generate comprehensive, multi-dimensional maps of the key genomic changes in major types and subtypes of cancer [26]. We would end this note by stating that LncRNAs in the future could be an ideal biomarker that has been the missing link in the management of the great malady with both diagnostic and therapeutic implications.

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