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SHORT COMMENTARY

ETS-FUSions Networking, Triggering and Beyond

Yue Zhang

Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA. Email: yzhang1@bidmc.harvard.edu

Abstract: Gene fusion is a hallmark of cancer development with the mechanisms underlying their genesis emerging. The Staege and Max paper together with another recent paper have provided a comprehensive first view on current TET-ETS translocation studies. This significance, the trigering of gene fusion and beyond will be discussed in this article.

Keywords: gene fusion, TET-ETS, networking, cell signaling, target gene, triggering, cancer stem cell, epigenetics, ncRNA

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Gene fusion is considered the driving force of cancer development with the mechanisms underlying their genesis emerging.[1] The Ewing sarcoma family of tumors (EFT) have a pronounced metastatic proclivity and are refractory to chemotherapy and radiation therapy. Elucidation of the genetics and epigenetics of the translocation network involving. TET (also FET, i.e. FUS, EWS, TAF15) and ETS family members, including the triggering of gene fusions, identification of their downstream targets, purification of cancer stem cells, generation of transgenic animal model and epigenetic mechanisms represents an important direction in understanding roles of the TET-ETS translocation network in mechanisms in cancer pathogenesis and developing potentially relevant therapeutic avenues in the future.

Two recent papers[2,3] have detailed the advances in understanding the TET-ETS translocation network with special focus on the Ewing family of tumors. These papers merge into a comprehensive first view on current EFT studies and therefore know where we are now and the challenge ahead. Staege and Max highlighted the core and extended TET-ETS translocation network and the frontiers of characterization of these fusions, i.e. the epigenetic mechanism in EFT. In addition, they demonstrated a clear aberrant mitosis in living EFT cells (i.e. SK-N-MC cells) and discovered two EWSR1 pseudogenes present in the human genome and this indicates that EWSR1 sequences have repeatedly been involved in rearrangements. Great progress in TET-ETS research has been made, but significant challenges remain.

TET-ETS Translocation Network

Staege and Max[2] also described a simple and clear core (i.e. translocations involving members of the TET family and/or ETS partners only) and extended TET-ETS translocation network (which includes other partners). Thus, scientists involved in research on different TET-ETS fusion research could benefit from a comparative analysis.

Target gene network and cell signaling pathways

As emphasized by Staege and Max and other experts, it is crucial to elucidate EWS/ETS target gene networks within the context of signaling pathways.[2,3]

A large array of direct target and indirect target genes of TET-ETS come to light by using RNA microarray and ChIP-chip and ChIP-seq (4) as well as proteomics approaches.[3]

Some of these networks are reproducibly identified and well characterized:[2–5]

1. Genetic up-regulated direct targets, such as EZH2, IGF1, etc;
2. Genetic down-regulated direct targets, like p21/CDKN1A, TGFBR2, IGFBP3;
3. Genetic up-regulated indirect targets, such as MYC, NKX2-2, CAV1, etc;
4. Genetic down-regulated indirect targets, like p27/CDKN1B, p57/CDKN1C, etc;
5. Proteomic up-regulated direct targets, like RPB7, HSP90, etc.

Interestingly, HSP90 inhibitor 17AAG can sensitize cells to the induction of programmed cell death by ionizing radiation and conventional chemotherapeutics,[7] so these results could suggest a beneficial effect of 17 AAG on intervention of EFT.

Importantly, many of these targets are in the main cell signaling pathways,[2,3] which are involved in EFT proliferation-survival, neural differentiation, Wnt pathways, tyrosine kinase pathways, MAPK, PI3K pathways, immune response, etc.

The formation of fusion genes has multiple oncogenic effects. Singular fusion occurs in tumors of the same type and the chimeric fusion involves TET and/or ETS family members. The chimeric EWSR1-FLI1 transcription factor binds not only ETS consensus sites but also microsatellite sequences and then regulates gene expression after binding to these microsatellite sequences.[4] Thus the target gene network of wild type seems be systematically disrupted by the combined action of the EWS transcriptional activator and the FLI1 DNA-binding domain, they function as either aberrant transcription factors or potent repressors, or by altering RNA processing. Finally, TET-ETS fusion proteins are not sufficient to induce the complete gene expression program of EFT. Surprisingly, EWSR1-FLI1 expressing transgenic animals did not develop EFT like sarcomas but leukemia.[2] Therefore, knowing the exact function of TET-ETS fusion proteins in cancer pathogenesis is far from complete.

Triggering of gene fusion

Of great importance is that fusion partners of TET and ETS family members are involved in additional chromosomal rearrangements. The inhibition of wild type EWSR1 function by EWSR1-FLI leads to mitotic defects. Staege and Max observed aberrant mitotic figures in cultured EFT cells and this might be responsible for the high frequency of secondary chromosomal aberrations in EFT.

In general, the fusions result from two double strand breaks which erroneously repair with incorrect DNA ends. The breakpoint region has Alu repeats, which is amenable to a recombination event.[8] Estrogen can induce rapid chromosomal movements that bring together estrogen receptor α-bound genes on different chromosomes. Similarly, LnCaP prostate cancer cells stimulated with the androgen receptor (AR) ligand dihydrotestosterone (DHT) induced proximity between TMPRSS2 and ERG genomic loci. This induced proximity facilitates the formation of gene fusions when irradiating the cells to further induce DNA double strand breaks.[1] Such triggering may take place in the genesis of TET-ETS fusion.

Cancer stem cells

Importantly, Staege and Max also noted that tumor stem cells in EFT have been identified. These tumor stem cells expressed some markers of embryonic stem cells. There are cell populations with the phenotype of embryonic stem cells in the adult body.[2,9,10] It remains unclear as to whether such cell populations are permissive for EWSR1-FLI1 induced transformation and whether EFT is derived from these cell populations.

Epigenetics and ncRNAs (microRNA or LincRNA)

Finally, the Staege and Max paper[2] brings attention to epigenetic mechanisms in EFT development. Epigenetics mediated by TET and/or ETS gene fusions play a major role in cancer pathogenesis. Some tumor suppressor genes have been epigenetically inactivated in EFT and inhibitors of histone deacetylation or DNA methylation can exert anti-tumor activity against EFT. Similarly, TMPRSS2 (transmembrane protease, serine 2)-ETS translocations in prostate cancer are associated with increased histone deactetylase expression. One of the target genes of EWSR1-FLI1, enhancer of zeste homolog 2 (EZH2), is involved in the epigenetic inactivation of genes. EZH2 is up-regulated in EFT which may inactivate the differentiation inducing genes.[2,3] Over-expression of epigenetic silencers like EZH2 may fix the tumor cells in an un-differentiated state. Indeed, inhibition of EZH2 allows differentiation of EFT cells and inhibits tumor growth.

The ncRNAs, *esp.* miRNAs and Linc RNAs also emerge as additional cellular epigenetic master regulators and they control a layer of gene expression.[7,11,12]

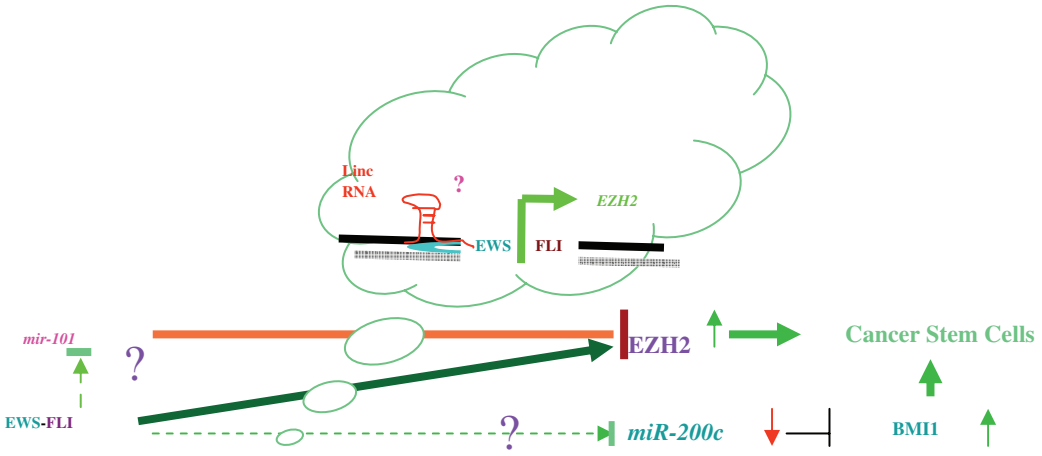

Figure 1. Hypothesized cross-talks between EWS-FLI and ncRNAs/EZH2 in EFT.
1. In Cloud: the ncRNAs or LincRNAs may bind and lead activator (or repressor) proteins to promoter and activate (or silence) gene expression.
2. EZH2 up-regulation keeps the tumor cells in an un-differentiated state.
3. *mir-200c* decreased expression in cancer stem cells compare to cancer cells in the bulk tumor. *mir-200c* might block stem cell self—renewal by targeting the self—renewal gene BMI1 protein. The dash line (and question) means further studies needed.

This could spur us to investigate the roles of ncRNAs in the cancer stem cells and development of EFT. Interestingly, *miR-101*, by virtue of its regulation of EZH2, may have profound control over the epigenetic pathways in cancer cells.[8] Over-expression of *miR-101* may change the histone code of cancer cells. Though the cross-talk between EWS-FLI and ncRNAs/EZH2 in EFT remain elusive (Fig. 1), it is now clear that the human TET proteins are associated with transcription, microRNA (miRNA) processing, etc.[2,3] In the future, studies of ribonomics with RNA immunoprecipitation and HITS-CLIP techniques[13] on EFT could reveal new roles of TET-ETS in the mysterious RNA world.

Disclosures

This manuscript has been read and approved by the author. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The author reports no conflicts of interest.

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