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CLINICAL IMPLICATIONS

C/EBPβ LIP induces a tumor menagerie making it an oncogene

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"But it has never been shown that endogenous C/EBPβ can function as an oncogene," so it goes in this issue's contribution by Bégay and colleagues [1]. Cytosine-cytosine-adenosine-adenosine-thymidine box-motif (CCAAT)-enhancer-binding proteins (or C/EBPs) are a transcription factor family composed of six members, named from C/EBPα to C/EBPζ. The C/EBP transcription factors promote the expression of certain genes through interaction with their promoters and enhancers. Once bound to DNA, C/EBPs can recruit so-called co-activators that in turn can open up chromatin structure or recruit basal transcription factors. C/EBP proteins interact with a palindromic CCAAT DNA sequence (reads the same forward or reverse), which is present in numerous gene promoters. The C/EBP transcription factors all feature a highly conserved basic-leucine zipper (bZIP) domain at the C-terminus. This domain is involved in dimerization and DNA binding, as are other transcription factors of the leucine zipper domain-containing family, such as c-Fos and c-jun. The bZIP domain structure of C/EBPs is composed of an α-helix that forms a "coiled-coil" structure when it dimerizes. Members of the C/EBP family can form homodimers or heterodimers with other C/EBPs and with other transcription factors in a network of extensive molecular crosstalk. C/EBP proteins also contain gene activation domains at the N-terminus and regulatory domains that respond to extracellular signals.

C/EBPβ is critical for normal macrophage functioning, an important immune cell subtype [2, 3]. Mice unable to express C/EBPβ have macrophages that cannot differentiate (specialize) properly and thus are unable to perform all their biological functions—including macrophage-mediated muscle repair [4]. Observational work has shown that expression of C/EBPβ in leukocytes is directly associated with muscle strength in humans, emphasizing the importance of the immune system and particularly macrophages, in the maintenance of muscle function [5].

C/EBPβ is involved in cell growth, proliferation, differentiation, apoptosis, senescence, and tumor tolerance. Thus, a role for altered C/EBPβ function in cancer is not unexpected. But can C/EBPβ function as an oncogene? Todaro and Heubner first coined the term "oncogene." Src was the first discovery, responsible for (Peyton Rous') chicken sarcoma, where it was harbored by a retrovirus. Bishop and Varmus at the University of California, San Francisco demonstrated that oncogenes were found in many organisms including humans. For this discovery, proving Todaro and Heubner's "oncogene theory," Bishop and Varmus were awarded the Nobel Prize in Physiology or Medicine in 1989. So much is offered for background and recognition. Then, there is the term "proto-oncogene," a normal gene which, when altered by mutation, becomes an oncogene that can contribute to cancer. Many proto-oncogenes provide signals that lead to cell division, while others regulate programmed cell death, or other vital cellular functions. We could not live without our proto-oncogenes.

The gene encoding the protein C/EBPβ is *Cebpb* (in the mouse), a single exon, no intron gene. Nevertheless, there are three isoform proteins of variable N-terminal length that are expressed from internal AUG start sites (Fig. 1). The two long isoforms (termed C/EBPβ LAP* and LAP) are both transcriptional activators. However, they differ by 21 N-terminal amino acid residues that entail chromatin regulatory capacity. The truncated C/EBPβ LIP isoform lacks the N-terminal 185 amino acid residues, removing the entire transactivation domain and part of the regulatory domain. C/EBPβ LIP is thought to counteract tumor suppressive functions of other C/EBP family members, suggesting C/EBPβ LIP as a potential oncogene [6, 7]. Excess C/EBPβ LIP may promote breast cancer metastases by interfering with TGFβ cytostasis or by

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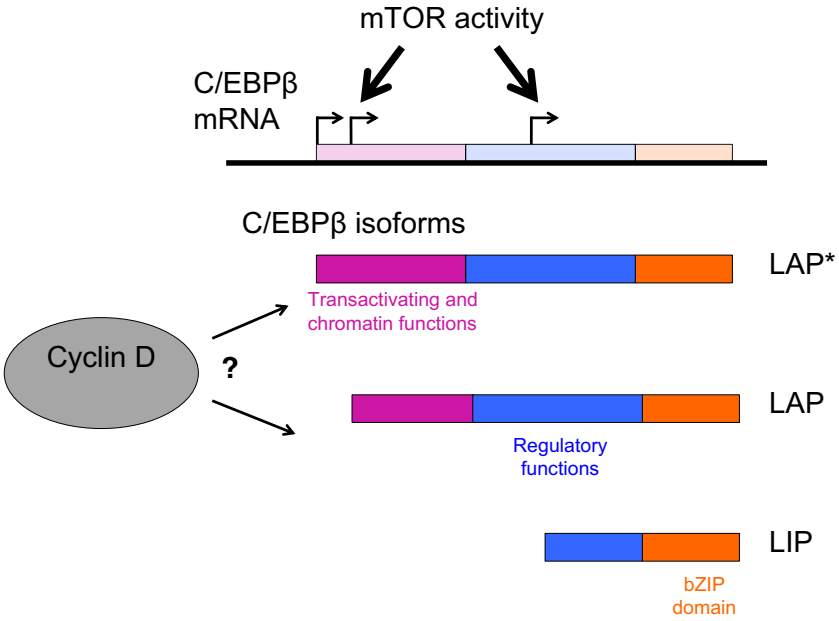

Fig. 1 The C/EBPβ gene does not possess introns and encodes three protein isoforms—referred to as LAP, LAP*, and LIP—generated by use of alternative in-frame translational initiation sites from a single mRNA. The concerted actions of the C/EBPβ isoforms participate in proliferation, differentiation, and cell fate determination in a number of different tissues. The ratios of LAPs to LIP are believed to influence biological outcomes brought about by C/EBPβ. According to Bégay and colleagues [1], deregulation of the LAP to LIP ratio (too much LIP) contributes to tumorigenesis

inhibiting anoikis [8, 9]. Anoikis is a form of programmed cell death, which is induced by anchorage-dependent cells detaching from the surrounding extracellular matrix. Cyclin D1, which is overexpressed in many tumors, may also bind to LAP, an event that modulates the transcriptional function of LAP in mammary epithelial-cell differentiation [10, 11].

Bégay et al. [1] used recombinant mouse genetics to explore whether or not deregulated expression of C/EBPβ LIP from its own genomic locus supports spontaneous tumor formation. They provide a knockin mouse strain that expresses the C/EBPβ LIP isoform but not the long isoforms LAP* and LAP. The mice developed tumors in multiple tissues of mesenchymal and epithelial origin, providing experimental evidence that enhanced translation reinitiation of C/EBPβ LIP is involved in tumorigenesis. Wild-type mice lived on average about 2 years, heterozygous C/EBPβ LIP mice 4 months less, and homozygous C/EBPβ LIP mice 7 months less than expected. About 35 % of C/EBPβ LIP mice had tumors, while about 9 % of wild-type mice did. Interestingly, the array of tumors was astounding. B cell non-Hodgkin's lymphomas, sarcomas, and adenocarcinomas were all represented. Impressive is the tumor array in C/EBPβ LIP heterozygous mice that included also T cell lymphomas, carcinomas of skin, liver, and mammary gland. The C/EBPβ LIP protein was highly expressed in lymphomas. The investigators studied deletion of *Cebpb* separately. *Cebpb* +/− mice lived as long as wild-type littermates and had no tumors. *Cebpb* −/− mice succumbed to infections.

To clarify mechanisms, the authors relied on gene-set enrichment analysis (GSEA). GSEA is a computational method that determines whether or not an a priori defined set of genes shows statistically significant, concordant differences between two biological states, for example phenotypes. The analysis drew attention to mTOR signaling. However, gene sets involved in translation and regulation of translation, mitochondrial function, metabolism, IGF1, and FOXO pathways were all significantly enriched in lymphomas of C/EBPβ LIP heterozygous mice. Diminished were gene sets involved in MAPK, ALK1, TGF−β, and NF-κB signaling survival pathways that could affect apoptosis. In short, the lymphoma analysis generally revealed an increase in pro-tumorigenic cytokine release, deregulation of chemokine expression, and toll-like receptor signaling pathways, in addition to reduced apoptosis, that could all predispose and contribute to tumor susceptibility.

Finally, the investigators tested whether or not deregulated C/EBPβ LIP expression promoted leukocyte precursor proliferation. They performed wild-type or C/EBPβ LIP homozygous bone marrow transplants into lethally irradiated mice. The bone marrow-transplanted mice exhibited more myeloid cells and less T cells than expected. The results are consistent with tumor supportive microenvironment in the C/EBPβ LIP cell-transplanted mice.

Where within C/EBPβ is this mystery and why (teleologically) must there by three C/EBPβ isoforms? An upstream open reading frame regulates alternative translation of C/EBPβ that restrains initiation of C/EBPβ LAP and causes resumption of ribosomal scanning and reinitiation at the downstream C/EBPβ LIP start site. The process depends upon mTOR signaling, stress pathways, and other influences. At high translation, LIP is produced; at lower translational requirements, LAP is in the foreground [12]. This idea suggests that the LIP/LAP ratio is a compelling factor, whether or not cancer will be produced by the nascent oncogene. The LIP/LAP relationship evidently plays a key role in liver regeneration, acute phase responses, bone homeostasis, and mammary gland development so that tumor development is not the primary aim but regeneration [13, 14].

Are there any surprises about C/EBPβ and its being an oncogene? Not hardly, since C/EBPβ has appeared in an article published in a journal of the same name [15]. Interestingly, the idea stems from the senior author of the present paper. Presumably, oncogenes (such as v-Src) develop through modifications of proto-oncogenes (c-Src) picked up by retroviruses. The proto-oncogene undergoes mutation in the process of becoming an oncogene. Over 20 years ago, the Leutz laboratory reported that the myeloid transcription factor (NF-M) is related to C/EBPβ and plays a role in signal transduction, differentiation, and leukemogenesis of avian myelomonocytic cells [16]. In this editorialist's bird's eye view, the laboratory first indicated that C/EBPβ could be a nuclear regulator connected to signal transduction molecules, such as protein kinase (proto-) oncogene products, which alter the activity of cellular transcription factors [17, 18].

The striking feature of the present report is the extensive panoply of tumors exhibited by these mice that extend from ectodermal, mesodermal, to endodermally derived tumors. The authors' primary interest is in hematological tumors. However, more information on the genesis of the other tumors would also be of interest.

Respectfully,
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