# ZEB Family

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Molecular signaling pathways involved in cancer have been intensively studied due to their crucial role in cancer cell growth and dissemination. Among them, zinc finger E-box binding homeobox-1 (ZEB1) and -2 (ZEB2) are molecules that play vital roles in signaling pathways to ensure the survival of tumor cells, particularly through enhancing cell proliferation, promoting cell migration and invasion, and triggering drug resistance. Importantly, ZEB proteins are regulated by microRNAs (miRs). In this review, we demonstrate the impact that miRs have on cancer therapy, through their targeting of ZEB proteins. MiRs are able to act as onco-suppressor factors and inhibit the malignancy of tumor cells through ZEB1/2 down-regulation. This can lead to an inhibition of EMT mechanism, therefore reducing metastasis. Also, miRs are able to inhibit ZEB1/2-mediated drug resistance and immunosuppression. Additionally, we explore the upstream modulators of miRs such as long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), as these regulators can influence the inhibitory effect of miRs on ZEB proteins and cancer progression.

Keywords: MicroRNA ; ZEB family ; Cancer therapy ; EMT ; Drug resistance ; Immunotherapy

## 1. Definition

Molecular signaling pathways involved in cancer have been intensively studied due to their crucial role in cancer cell growth and dissemination. Among them, zinc finger E-box binding homeobox-1 (ZEB1) and -2 (ZEB2) are molecules that play vital roles in signaling pathways to ensure the survival of tumor cells, particularly through enhancing cell proliferation, promoting cell migration and invasion, and triggering drug resistance. Importantly, ZEB proteins are regulated by microRNAs (miRs).

## 2. Introduction

Epithelial-mesenchymal transition (EMT) process was first introduced by Greenburg and his colleagues in 1982 [1]. To date, three major types of EMT have been identified: type I EMT, which occurs during embryogenesis, type II EMT, which is activated during wound healing, tissue regeneration and organ fibrosis, and type III EMT, which occurs during metastasis of cancer cells [2]. EMT is the process of cellular transition wherein epithelial cells are bio-transformed into mesenchymal cells with fibroblast-like properties [3][4][5][6]. In the EMT mechanism, cadherins play a significant role. Cadherins promote cell-cell adhesion and are located at the adherens' junctions. There are different kinds of cadherins including E, N, P, VE, proto, desmosomal, and FAT cadherins, but N-cadherin and E-cadherin are the most important ones in EMT mechanism. A decrease in E-cadherin levels, and an increase in N-cadherin levels lead to stimulation of EMT, and enhanced migratory ability of cancer cells [7][8]. Additionally, upon EMT stimulation, morphology changes and alterations in cytoskeleton occur in cells and affect their migratory ability and adhesion to neighboring cells. These molecular and structural changes promote the dissemination of cells into other sites [9]. Essentially, this increased cell migration is beneficial in normal cells to accelerate physiological processes such as wound healing and embryogenesis. It has been reported that EMT occurs to provide the required flexibility for mesoderm and neural crest formations [10][11]. However, cancer cells can exploit the EMT mechanism for metastasis to distant sites [12][13][14]. There is increased attention towards the EMT mechanism in cancer therapy not only because of its contribution toward metastasis, but also due to the fact that the EMT mechanism can trigger chemoresistance of cancer cells, and decrease sensitivity to apoptosis [15][16]. Therefore, understanding the molecular pathways regulating EMT is a crucial in the field of cancer studies.

EMT is regulated by a variety EMT-promoting transcription factors (EMT-TFs) such as Snail, Slug, Twist, TBX-2, SIX, transforming growth factor--β (TGF-β), and Zinc finger E-box-binding homeobox protein (ZEB) [17]. These upstream EMT-TFs can induce EMT and promote the biotransformation of cells from epithelial phenotype into mesenchymal phenotype by affecting levels of cadherins. Different studies have shown the involvement of ZEB proteins in modulating EMT during

normal development and in pathological conditions [18][19][20][21]. Our aim in the present review is to 1) show that ZEB proteins are able to regulate metastasis of cancer cells via affecting EMT, 2) understand how different microRNAs (miRs) can regulate the ZEB/EMT axis, and 3) demonstrate how other upstream mediators can regulate the miR/ZEB/EMT axis.

## 3. ZEB Family

The ZEB family, which was first discovered in Drosophila melanogaster, consists of two key members ZEB1 and ZEB2 [22]. Both ZEB1 and ZEB2 possess the amino-terminal (NZF) and carboxy-terminal zinc finger cluster (CZF), thereby allowing them to bind to regulatory DNA sequences in their target promoters [23][24][25]. This has led to their involvement in different biological events, such as embryogenesis, hematopoiesis, and more importantly, EMT. In fact, ZEB proteins are well-known due to their ability in stimulation of EMT [20]. In this section, we provide an overview of ZEB1 and ZEB2 proteins to shed some light on their role in cancer cells.

### 3.1. ZEB1

ZEB1 gene is located on chromosome 10p11.2, and its protein is made up of two zinc-finger clusters at N- and C-terminal ends, while the middle portion of the ZEB1 protein contains three distinct parts including a homeodomain, a Smad interaction domain and a C-terminal binding protein (CtBP). The CtBP is involved in the regulation of ZEB1 function [26][27]. Primarily, the zinc-finger clusters allow ZEB1 to bind to E-boxes. ZEB1 regulates its downstream effectors through binding to E-promoter DNA sequence (5′-CANNTG-3′) [28]. Various publications have also highlighted ZEB1′s association with enhanced viability and invasiveness of cancer cells. In colorectal cancer (CRC) cells, it was found that tumor suppressor death domain-associated protein (DAXX) is able to prevent ZEB1 modulation on E-cadherin to inhibit the invasion and proliferation of tumor cells. Down-regulation of DAXX enhanced ZEB-1 suppression of E-cadherin, leading to the enhanced proliferation and malignancy of cancer cells [29]. It has also been highlighted that EMT may contribute to chemoresistance of cancer cells [30][31]. In pancreatic cancer, Rho associated coiled coil containing protein kinase 2 (ROCK2) enhances the expression of ZEB1. This in turn leads to ZEB1-mediated EMT induction, which contributes to gemcitabine resistance in pancreatic cancer cells [32]. In CRC cells, TCF4 enhances expression of ZEB1 to promote stemness and migration of cancer cells, thereby promoting chemotherapy resistance [33]. In prostate cancer cells, ZEB1 stimulates up-regulation of ATP-binding cassette subfamily C member 10 (MRP4) to export docetaxel out of cancer cells, resulting in their decreased sensitivity to chemotherapy [34]. These studies support the modulation of ZEB1, and highlights that it may be beneficial in enhancing the efficacy of chemotherapy and in reducing the migratory ability of cancer cells. Overall, ZEB1 is an important mediator to enhance the invasion and proliferation of tumor cells. More importantly, ZEB1 may significantly reduce the efficiency of chemotherapy.

### 3.2. ZEB2

ZEB2 is another member of ZEB family and is located on chromosome 2q22.3 [25]. Structurally, the N-terminal end of ZEB2 consists of four zinc fingers, while the C-terminal end has three zinc fingers [25]. Similar to ZEB1, ZEB2 appears to play a crucial role in migration and invasion. In non-small cell lung cancer (NSCLC), MDM2 binding protein (MTBP) behaves as an oncogene to increase EMT through ZEB2 up-regulation [35]. This in turn enhanced the migration and metastasis of NSCLC tumor cells. In bladder cancer, it was found that indoleamine-2,3-dioxygenase-1 (IDO1) induces ZEB2 overexpression, which in turns increases the viability and proliferation of cancer cells [36]. ZEB2 has also been found to increase the expression of ETS proto-oncogene 1 (ETS1) to up-regulate other EMT proteins such as matrix metalloproteinase 9 (MMP-9) and Twist [37]. Importantly, ZEB2 is also capable of inducing chemoresistance via EMT activation. Phosphatidylinositol 3-kinase (PI3K)/protein kinase-B (Akt) pathway is a down-stream pathway of ZEB2 that induces EMT by reducing the level of E-cadherin protein, leading to the generation of cisplatin resistance in NSCLC cells [38] In all, ZEB2 appears to mediate EMT, and may be a potential therapeutic target in cancer treatment.

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