The importance of epithelial-mesenchymal transition and autophagy in cancer drug resistance

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Abstract

Epithelial-mesenchymal transition (EMT) and autophagy are both known to play an important role in the development of cancer. Subsequently, these processes are now being utilised as targets for therapy. Cancer is globally one of the leading causes of death, and despite many advances in treatment options, patients still face many challenges. Drug-resistance in cancer-therapy is a large problem, and both EMT and autophagy have been shown to contribute. However, given the context-dependent role of these processes and the complexity of the interactions between them, elucidating how they both act alone and interact together is important. In this review, we will provide an insight into the current landscape of the interactions of autophagy and EMT in the context of malignancy, and how this ultimately may affect drug-resistance in cancer-therapy.

Epithelial-Mesenchymal Transition (EMT)

Epithelial-mesenchymal transition is an important biological process which is critical in developmental biological and wound healing, but has also been implicated in fibrosis and malignancy (1-4). It is a reversible, biological process associated with loss of cell polarity and cadherin-mediated cell adhesion in epithelial cells. These cells transition to mesenchymal cells and in turn, gain migratory and invasive abilities (5). EMT is mediated through a number of signalling pathways, including TGF- β, Wnt-β-catenin, Hedgehog, Notch, Bone Morphogenetic Protein (BMP) and receptor tyrosine kinases (6). Signalling pathways in turn mediate EMT specific transcription factors (EMT-TFs) such as ZEB1/2, Snail1/2 and Twist, which subsequently act to repress the expression of target genes, including E-cadherin, loss of E-cadherin is considered a key step in EMT (6-9). In the context of malignancy, EMT can result in cells metastasizing from primary tumour sites, which has been associated with a worse prognosis (Figure 1).

Autophagy

Autophagy is an evolutionarily conserved, biological process where long-lived proteins and damaged-organelles are degraded by the lysosome (10, 11). There are two types of autophagy: general and selective autophagy; in general autophagy part of the cytoplasm is engulfed, which is delivered to the lysosome and this is degraded. (Macro) autophagy is where a double membraned vesicle is formed, which captures material in the cytoplasm to be degraded. Whereas selective autophagy, specifically targets cargo to be degraded (12-15). Autophagy has also been proposed to have roles in a number of diseases (16), such as fibrosis (17-19), neurodegeneration (20) and cancer (21, 22) (Figure 2).

The role of autophagy in malignancy is complicated, with conflicting reports on the role of autophagy in a number of different contexts (23-27). It is thought that autophagy largely aids in tumour suppression in early tumorigenesis, whereas it can promote tumour progression and cancer-cell survival in the later stages. It is understood that autophagy is able to prevent the formation of tumours by maintaining stability in normal cells. During early stages, autophagy protects normal cells from transforming by preventing genomic instability, and thus preventing formation of an inflammatory microenvironment. In comparison, in the later stages, autophagy helps survival of cancerous cells undergoing a number of cellular stresses such as metabolic stress and preventing cell death by anoikis (28, 29).

Increased autophagy has been associated with cancer as a mechanism to aid survival and resist treatment (30), with tumours being shown to require autophagy for survival (31, 32). As such, autophagy inhibitors have been utilised both alone and in combination with traditional therapy. With a number of studies demonstrating that autophagy inhibition is able to sensitise cancer cells to further treatment (33-35).

EMT and Autophagy: A complex relationship in malignancy

The signalling pathways of both EMT and autophagy are complex and can be induced in a number of ways, it is therefore unsurprising that there is some interaction between these two pathways (36, 37). Numerous studies in a number of contexts have demonstrated interactions between autophagy and EMT, although it does appear this is both context- and tissue-dependent (Table 1). A number of studies have shown that manipulation of autophagy, was able to promote EMT, invasion and metastasis (21, 27, 38-40), these have been demonstrated in a wide variety of tissues/cell lines including pancreatic, breast, colorectal, melanoma and gastric. 1,400 tumours from 20 different types of cancers were analysed for LC3B, an

autophagy marker, it was found that increased expression was associated with metastasis and invasion (27). Autophagy inhibition in RAS-mutant cancer cells was demonstrated to induce EMT by triggering NF-κB by p62/SQSTM1 (21). Similarly p62/SQSTM1 is important in stabilising Twist1, preventing its degradation (40). In gastric cancer cells, autophagy inhibition promotes EMT and alters the metabolic phenotype of cells, and this is dependent on ROS- NF-κB-HIF-1α (38). Beclin-1 has shown in colon cancer cells to be associated with EMT and invasive behaviours, loss of Beclin-1 was able to reverse this phenotype (41). As described the role of autophagy is dual: in pancreatic ductal adenocarcinoma (PDAC) cells, TGFβ1 induced autophagy in SMAD4-postive cells and inhibited migration by reducing nuclear translocation of SMAD4, whereas in SMAD4-negative cells migration was increased through MAPK/ERK (42).

Manipulation of autophagy has also been demonstrated to prevent an EMT-like phenotype and associated metastasis/invasion in a number of cancer cell lines and tissues including breast, colorectal, pancreatic and ovarian cancers (42-48). In hepatocellular carcinoma (HCC) autophagy inhibition was not shown to induce EMT and had no effect on migration or invasion (43). Death effector domain-containing DNA-binding protein (DEDD) attenuates EMT by interacting with BECN1 and PIK3C3 activating autophagy (44). In ovarian carcinoma, danusertib induced autophagy which resulted in suppression of EMT and arrest of G2/M phase and this may be in part due to P13K/Akt/mTOR signalling (45). Similarly FAT4 has been shown to regulate activity of PI3K to induce autophagy and inhibit EMT (49).

Given the complicated role of autophagy in malignancy and how a number of clinical trials are now utilising autophagy inhibitors as treatments for cancer

(http://www.cancer.gov/clinicaltrials), the wider-reaching implications of these drugs needs to be further investigated.

Drug Resistance – Where does EMT come into play?

Drug resistance is a well-known concept where diseases become unaffected by pharmaceutical treatment, which has been studied in a variety of disease models. Two types of drug resistance have been described; acquired and de novo (50). Initially many cancers can be treated with 'conventional' therapies such as chemotherapy, however as the biochemical and tumour environment adapt overtime, sometimes cancer cells become resistant to these treatments. This resistance can be due to many factors, not limited to: drug efflux, metabolism, changes in drug target, DNA damage repair, cell death inhibition and EMT (51).

The link between EMT and drug resistance in cancer was proposed in the 90s (52) and subsequently a number of different cancers have reported drug resistance to be associated with EMT, including lung (53), pancreatic (54, 55), bladder (56) and breast cancer (57, 58). Activation of a number of signalling pathways known to induce EMT, such as TGFβ, Wnt, Hedgehog (Hh) and Notch (59-62) have also been demonstrated to induce cancer drug. Some of the specific mechanisms have begun to be elucidated, but due to the large variety of drugs, tissue types and different signalling pathways involved it is a complex process, as summarised in Table 2.

TGFβ signalling has been implicated in a number of different tissues including colorectal, breast and squamous cell carcinoma stem cells (59, 63-66), although mechanistically its involvement in drug resistance has been varied. Some studies demonstrated a role for metabolism, showing that TGFβ regulated 5-FU resistance in CRC through the regulation of

Pyruvate Dehydrogenase Kinase 4 (PDK4) (66). In squamous cell carcinoma, TGFβ transcriptionally activates p21, this stabilises NRF2 which enhances glutathione metabolism and reduces the effectiveness of therapies (64). Conversely doxorubicin (Dox) resistance in colon cancer, which was also demonstrated to be via TGFβ-upregulation further when Smad4 was downregulated it was able to increase sensitivity of cells to Dox (59). In Triple Negative Breast Cancer (TNBC) TGFβ was shown to be critical in epirubicin-resistance by regulating EMT and apoptosis (63). Long term TGFβ treatment has also been associated with anti-cancer drug resistance (65).

A number of other EMT-inducing pathways have also been directly linked to drug-resistance in cancer. The Wnt has been demonstrated to cause drug resistance in HER2-over expressing breast cancer, Type-1 epithelial ovarian cancer (EOC) and gastric cancer (60, 67, 68). In HER2-overexpressing breast cancer cells, it is considered that Wnt3 overexpression may activate Wnt/β-catenin transactivating EGFR, which can lead to a partial-EMT which could be important in understanding trastuzumab resistance in these cells (60). In EOCs, Dapper1 Antagonist of Catenin1 (DACT1) has been shown to negatively regulate Wnt signalling and regulate cis-platinum resistance, through regulating autophagy. EOC cells transfected with a lentivirus carrying full-length DACT1, had increased levels of autophagy and were more sensitive to cisplatin (67). In gastric cancer, NANOGP8 overexpression leads to anti-oxaliplatin (L-OHP) resistance. It upregulates EMT markers and increases β-catenin accumulation in the nucleus and strengthens Wnt signalling (68). Activation of the Hedgehog (Hh) pathway has also been linked to drug resistance in both non-small cell lung cancer (NSCLC) with resistance to EGFR-TKIs (61), and in CRC with resistance to cetuximab (69). Finally, the Notch pathway has been implicated in drug resistance in pancreatic cancer. Both Notch-2 and its ligand Jagged-1 are upregulated in gemcitabine-

resistant cells and knockdown of Notch resulted in partial-reversal of EMT characteristics (62).

Numerous studies in a variety of tissue types have also found EMT-TFs; SNAIL1/2, ZEB1/2 and TWIST to directly confer to drug-resistance in cancer (70-81), summarised in Table 2. Upregulation of these transcription factors alone can be sufficient to confer to drug resistance (71, 75). ZEB1 is highly expressed in glioblastoma cells, where a ZEB1-miR200 feedback loop connects this with a number of downstream targets (ROBO1, c-MYB, MGMT), and increased levels of this EMT-TF are associated with both drug-resistance and reduced survival (79). In CRC, the FBXW7-ZEB2 axis has been demonstrated to control a number of important EMT associated characteristics as well as drug-resistance. ZEB2 knockdown was able to reverse the EMT-phenotype induced by loss of FBXW7, a tumour suppressor (81). Similarly, over-expression (70) or up-regulation (71, 73) of TWIST has resulted in chemo resistance in cancer cells; mechanistically in bladder cancer this has been shown to be through the upregulation of P-Glycoprotein (72). A number of EMT-TF including TWIST, SNAIL, FOXC2 have been shown to increase levels of ABC transporters, these are overexpressed in cancer and can remove cytotoxic drugs, and therefore increased levels confer to drug resistance (77, 78). In cisplatin-resistant cell lines, both morphological and phenotypic hallmarks of EMT were identified, gene expression profiling identified a number of EMT-TF, including Snail1/2 which were further validated as key players in drug resistance (74). These EMT mechanisms have been demonstrated in a wide-variety of cell lines/tissues including colon, breast, ovarian, gastric and glioblastoma cells, and with a number of different drugs, suggesting a significant issue.

Autophagy and Drug resistance

Autophagy has been implicated in drug-resistance in malignancy; chemotherapeutic agents have been shown to be limited in their capacity, as they were shown to induce protective-autophagy; and subsequently cancer cells became chemo-resistant. Cisplatin, a commonly used platinum compound for the treatment of a number of cancers, including ovarian cancer, induces autophagy via ERK and this confers to drug-resistance in these cancer cells (82). Further, inhibiting autophagy sensitised cancer cells to cisplatin-treatment (83, 84), with similar results also found in lung cancer (85). In esophageal cancer, cisplatin induced autophagy through the class III PI3K pathway and when cisplatin was used together with autophagy inhibitor 3-MA it augmented the affect of the treatment, compared to cisplatin alone (86).

Another example of this is 5-Fluorouracil (5-FU) which acts by inhibiting DNA synthesis (87), although its ability is ultimately limited as it induces autophagy in cancer cells which leads to chemo-resistance. A number of autophagy-related genes have been linked to multi-drug resistance in colorectal carcinoma (88). Blocking autophagy was able to sensitise cancer cells to 5-FU mediated death (89, 90). JNK activation and phosphorylation of Bcl-2 have been demonstrated as key components in 5-FU induced-autophagy in colon cancer (89), where 5-FU induced autophagy protects cancer cells (87). Similar findings have been shown in gallbladder carcinoma where 5-FU also induced autophagy, and inhibition of autophagy with chloroquine (CQ) was able to kill cancer cells (91). Similar findings have been demonstrated in a range of other cancers, including ER-positive breast cancer where autophagy inhibition can re-sensitize breast cancer cells to tamoxifen (92). In prostate cancer, high levels of Nitrogen Permease Regulator-Like 2 (NPRL2), a tumour supressor candidate gene, can cause resistance to Everolimus by enhacing autophagy, via mTOR (93).

Aoptosis and autophagy are closely linked processes and often involved in crosstalk, and it is thought that drug-induced autophagy can protect cancer cells from apoptosis. In breast cancer cells treatment with Epirubicin (EPI) induced autophagy in MCF-7 cells and this protected them from drug-induced apoptosis. In the drug-resistant MCF-7 cells, autophagy inhibition was able to re-sensitise cells to treatment (94). Finally, three common chemotherapeutics used in the treatment of osteosarcoma induced upregulation of HSP90AA1, which was shown to be a regulator of autophagy via PI3K/Akt/mTOR and apoptosis via JNK/p38 (95). Understanding the crosstalk of these pathways in the context of drug resistance will be critical in the development of new therapies.

Given that autophagy and EMT appear to have a complex relationship in malignancy; and that EMT has been demonstrated to contribute to drug resistance, a greater understanding of these relationships is key. New therapeutic strategies are being developed to try to target drug-resistance and targeting autophagy using inhibitors is one of the methods proposed and was able to sensitise cells to chemotherapy (96-103). Anti-cancer drugs have increasingly been utilised in combination with autophagy inhibitors. When cisplatin was used in combination with autophagy inhibition, this increased cytotoxicity in cells (48, 104). Similarly, the effects of 5-FU are augmented in colon cancer when treated with autophagy inhibitor hydroxychloroquine (HCQ)(105).

Conclusions and future directions

It is clear the underlying mechanisms in cancer drug resistance are multifaceted with a number of complex-interacting signalling pathways and processes contributing to resistance. These mechanisms are often highly specific depending on tissue-type and stage of disease. Although understanding the implications of these drugs alone on drug-resistance is being better elucidated, understanding how these processes interact and the effect this may have on treatment is limited. In many cancers, autophagy inhibitors are being utilised with traditional therapies which can increase cytotoxicity of the drugs. Further anti-cancer drugs can become resistant through an up-regulation of autophagy. However, autophagy inhibition in malignancy has been associated with EMT. Clinically EMT has been linked to cancer-drug resistance in addition to EMT-inducers and EMT-TF. In order to best optimise treatment it seems therapies needs to be in combination and targeted, and be tissue-specific.

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Figure Legends

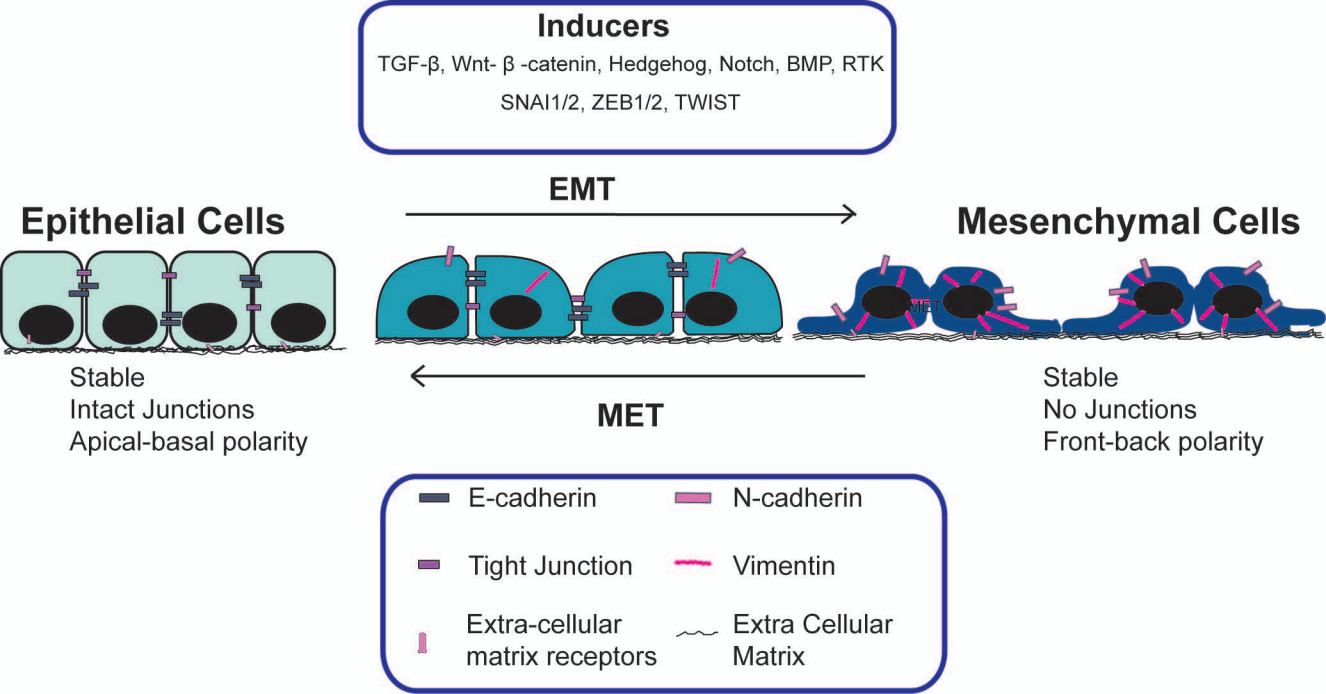
Figure 1 The role of Epithelial Mesenchymal Transition (EMT) in malignancy, with key biological features and EMT-inducers highlighted.

Figure 2 Role of autophagy in cancer: the formation of a double-membrane to engulf material to be degraded by the lysosome by autophagy. The role of autophagy in cancer is complex and has both a pro- and anti- tumour effect. Further discussion in review.

Tables

Table 1 Autophagy and EMT: dual role in cancer. Autophagy has been described to be both pro- and anti- tumour, highlighted are some of the recent works in a variety of tissue- types where the dual role of autophagy in malignancy has been demonstrated.

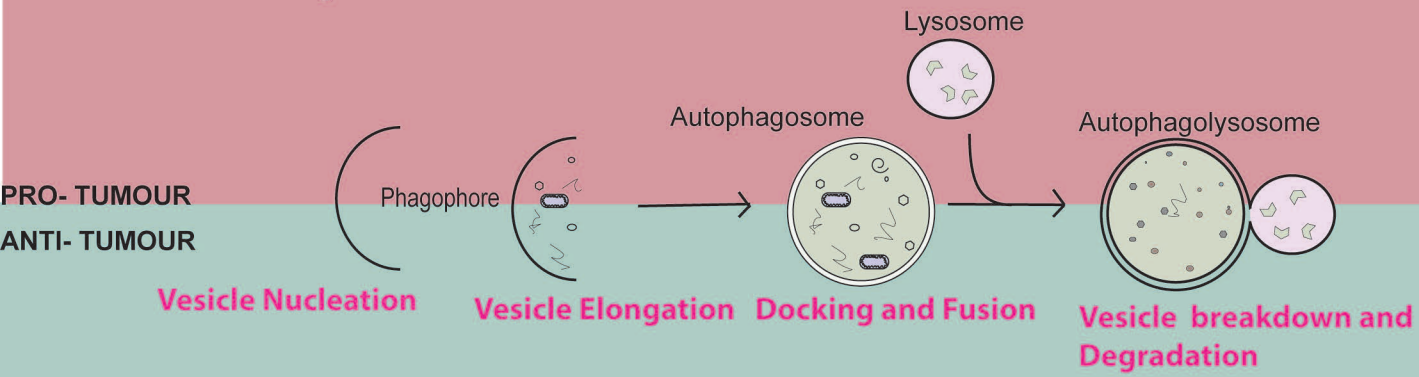
Table 2 EMT signalling pathways and EMT-TFs – contribution to drug resistance. A number of recent studies describing EMT pathways and transcription factors which have been demonstrated to be involved in drug resistance in cancer.


Inducers
TGF-β, Wnt- β -catenin, Hedgehog, Notch, BMP, RTK
SNAI1/2, ZEB1/2, TWIST
EMT
Epithelial Cells
Mesenchymal Cells
MET
Stable
Intact Junctions
Apical-basal polarity
Stable
No Junctions
Front-back polarity
E-cadherin
N-cadherin
Tight Junction
Vimentin
Extra-cellular matrix receptors
Extra Cellular Matrix

Aids survival under metabolic stress

Prevents cancer cell death

Drug Resistance



Helps normal cell homeostasis

Prevents genomic instability

Prevents formation of chronic inflammatory microenvironment

Table 1

Promotes EMT			
Autophagy	**Role on EMT**	**Cell/ Tissue type**	**Study**
Inhibition of autophagy	Promotes metastasis through induction of ROS	Gastric cells	(38)
Increased LC3B expression	Associated with metastasis	Breast cancer, melanoma and 18 other cancers	(27)
Autophagy inhibition (ATG KD, histology)	Promotes EMT and invasion	Colorectal cancer, pancreatic cancer (in RAS-mutated cells)	(21)
Autophagy induced by TGFβ	Promotes EMT	NSCL	(39)
Autophagy inhibited (ATG KD)	Promotes EMT via Twist	MEFs, keratinocytes, melanoma cells	(40)
Autophagy induced by TGFB	Inhibited proliferation, increased migration by MAPK/ERK activation	Pancreatic cancer (SMAD4-neg)	(42)
Inhibition by BECN1	Prevents EMT	Colorectal cancer	(41)

Prevents EMT			
Autophagy	**Role on EMT**	**Cell/ Tissue type**	**Study**
Autophagy inhibition	Prevents metastasis	Hepatocellular carcinoma	(43)
Activated autophagy	Prevents EMT by Snail and Twist	Breast	(44)
Autophagy induced by TGFB	Inhibited migration, promoted proliferation	Pancreatic cancer (SMAD4-pos)	(42)
Autophagy induced by Danusertib (pan-inhibiter of Aurora kinases)	EMT inhibited, potentially via PI3K/Akt/mTOR	Ovarian carcinoma	(45)
Increased autophagy by overexpression of FAT4	Prevents EMT. Regulatory effects of FAT4 on autophagy and EMT are partially by PI3K-AKT	Colorectal cancer	(46)
Increased autophagy	Protects cells from anoikis, promoting luminal filling in early carcinoma	Breast cancer	(47)

Table 2

	Mechanism of resistance	Tissue type	Study
Signalling pathway			
TGFβ	Upregulation of TGFβ	Colon cancer cells	(59)
	-	Triple Negative Breast Cancer	(63)
	-	Squamous cell carcinoma stem cells	(64)
	-	Breast cancer cells (HMLER)	(65)
	Regulating the expression of pyruvate dehydrogenase kinase 4 (PDK4)	Colorectal cancer	(66)
Wnt	Trastuzumab resistance associated with Wnt3 overexpression activates Wnt/ β -catenin which transactivates EGFR	HER2-over expressing breast cancer	(60)
	Resistance to platinum-based chemotherapies. Dapper1 Antagonist of Catenin 1 (DACT1) demonstrated to be a negative regulator in EOC, inhibiting Wnt signalling and cis-platinum resistance through regulation of autophagy	Type I epithelial ovarian cancer (EOC)	(67)
	NANOGP8 is main regulator. It is closely related to EMT and the Wnt pathway, and correlates with migration, invasion and chemo resistance in gastric cancer.	Gastric Cancer cells	(68)
Hedgehog (Hh)	Hh pathway activated in EGFR-WT and EGFR-mt lung cancer	Non-small cell lung cancer (NSCLC)	(61)
	HH pathway activation EGFR and EPHB3 crosstalk through HH-STAT3. But loss of HH may result in cells being more EGFR-dependent	Colorectal Cancer	(69)
Notch	Activation of Notch signalling.	Pancreatic cancer	(62)

	Mechanism of resistance	Tissue type	Study
EMT-TF			
TWIST	-	Colorectal carcinoma	(70)
	TWIST upregulation	Nasopharyngeal carcinoma	(71)
	Activated Twist mediates P-glycoprotein expression	Bladder cancer	(72)
	-	Breast cells	(73)
Snail1/2	-	Ovarian adenocarcinoma	(74)
	-	High grade Serous ovarian cancer (HGSOC)	(75)
	-	Oral squamous cell carcinoma	(76)
	ABC transporters are overexpressed in cancer, these are able to remove cytotoxic drugs by ATP-dependent efflux. EMT-TF such as **TWIST, SNAIL and FOXC2** demonstrated increased levels of ABC transported, which was directly related to drug resistance.	Breast	(77, 78)
ZEB1	ZEB1-miR200 feedback loop. ROBO1, OLIG2, CD133 and MGMT identified as novel ZEB1 targets.	Glioblastoma	(79)
	Increased IL-1β increases ZEB1 and was associated with increased resistance	Colon Cancer	(80)
ZEB2	Loss of FBXW7	Colorectal cancer	(81)