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Review

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Targeting epithelial-mesenchymal transition - an ongoing wild goose chase

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Abstract

Epithelial-mesenchymal transition (EMT) is a natural phenomenon thatoccurs during embryodevelopment. It is a phenomenon involving the transition of adherence-dependent stationary epithelial cells to adherence-independent migratory mesenchymal cells. Tumours reactivate this machinery and evade anti-tumour immunity and inhibition by cancer-specific drugs. EMT harnesses complex crosstalk among cancer cell signalling pathways that make it difficult to tackle therapeutically, and it plays a pivotal role in cancer metastasis. Most screening platforms and approved drugs are limited by their applicability to epithelial cancers. There is a significant need for developing new strategies targeting metastatic cancers. Here, we review the challenges with the current methods of screening and available drugs for EMT and shed some light on the key essentials needed for next-generation drug discovery attempts.

Keywords: Epithelial-mesenchymal transition, high-throughput screening, drug discovery, drug resistance, tumorspheres, organoids

INTRODUCTION

Epithelial-mesenchymal transition (EMT) is considered as a major phenomenon which contributes to cancer metastasis and drug resistance[1], and many reviews are available for understanding the natural functions of EMT and its role in cancer progression[2-4]. Our primary goal here is to discuss the drug screening methodologies developed by various groups and their limitations, while surrendering to the

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factual status of EMT's vital role in tumour biology, but is not declared to be the only mechanism for drug resistance. In this review, we start with a brief introduction of EMT, the signalling pathways involved in EMT, and the role of EMT in drug resistance, concluding with the need for developing new screening methodologies to overcome the limitations of the existing modalities.

OVERVIEW OF EMT

Epithelial cells are a differentiated type of cells that constitute the outer lining of human body organs, skin, urinary tract, and blood vessels. Mesenchymal cells originate from a type of connective tissue (called mesenchyme) found during embryonic development and therefore are understood to be stem cells. During embryogenesis, the process of EMT and mesenchymal to epithelial transition (MET) is a common observation[5,6]. EMT is not expected once the epithelial cells reach terminal differentiation[7,8], but the process is induced during tissue repair and unusual pathological stress[7,9]. Mostcancers arise from epithelial cells and grow indefinitely at that location. Epithelial cells interact laterally with other cells through junctional complexes and with the basal membrane via integrin receptors. These contribute to their apicobasal polarity and it is impossible to make them grow in isolation[10]. Some of those epithelial cells undergo EMT, lose cell-cell junctions, and acquire mesenchymal phenotype, which makes them independent of these constraints[11,12]. Having done this, these cells can migrate to other tissues, undergo MET, and establish successful metastases[6]. Benign tumoursof epithelial origin are not very harmful to the organism, but these metastases, especially when they are present in crucial tissues, are known to cause 90% of the mortality associated with these cancers[13]. The role of EMT in metastasis is not clearly established. In fact, Fischer *et al.*[14] showed that EMT is not required for successful breast to lung metastasis, as inhibition of EMT by inhibiting microRNA miR-200 did not inhibit the development of lung metastasis. EMT's role in developing chemoresistance, however, is indisputable[14].

CELL SIGNALLING INVOLVED IN EMT

EMT has been an untargeted pathway of cancer progression resulting in significant mortality and morbidity in multiple cancers. This has led researchers internationally to focus investigations on EMT targeting pathways in the cell. EMT is a multistep process whereby epithelial marker genes are suppressed, and mesenchymal markers are upregulated. The most important epithelial marker is E-cadherin. The transcription factors which can suppress its transcription are Snail1, Snail2 (also called Slug), zinc finger E-box-binding homeobox 1 (ZEB1), and ZEB2[15-17]. Twist, which is a master regulator of embryonic morphogenesis, is found to be essential for metastasis. High levels of Twist are observed in aggressive cancers and are associated with decreased E-cadherin[18]. The lymphoid-enhancing factor is needed for EMT induced by transforming growth factor-β3 (TGF-β3)[19]. Owing to their defined role in the process, all these transcription factors are termed EMT transcription factors (EMT-TFs). Recently, it was realised that EMT-TFs play key roles in almost all stages of cancer, i.e., initiation, primary tumour formation, invasion, dissemination, metastasis, and colonization at the secondary site[4]. Above all, the expression of the combination of EMT-TFs is different in different cancers and even within a single tumour. Thus, the markers determining the stages of EMT change, leading to the possibility of false-positive or false-negative results in disease prognosis testing[20,21]. Most of the transcription factors are regulated by receptor tyrosine kinases[22], TGF-β family[23], Wnt[24], Notch[25], and Hedgehog[26] pathways. The activators of these pathways include ligands such as EGF, TGF-β, cytokines, tumour hypoxia, and components of the extracellular matrix[27]. Of all these inducers, the TGF-β family has been most studied in the context of EMT. It consists of the TGF-β superfamily of ligands which include isoforms of TGF-β (TGF-β1, β2, and β3) and bone morphogenetic proteins[27]. The addition of exogenous TGF-β induces EMT in many cancer cell lines and is cell type-specific. Conversely, specific inhibition of TGF-β receptor-1 (TGF-βR1) abrogates this EMT induction[28]. As EMT is activated by these growth factors and transcription factors, most of the drugs targeting EMT affect these signalling pathways[29].

THE ROLE OF EMT IN TUMOUR IMMUNE EVASION AND DRUG RESISTANCE

EMT is a crucially important causative factor in tumour immune evasion and drug resistance. EMT has been shown to suppress cytotoxic T-lymphocyte mediated immunity in MCF7 cells by activating autophagy instead of apoptosis[30]. EMT-activated MCF7 cells express high levels of programmed death-ligand 1 (PD-L1) which contributes to suppression of tumour immunity. A ZEB1 transcription factor is important in this process and miR200 family members negatively regulate it[31]. EMT-related gene expression contributes to immunotherapy resistance against programmed cell death protein 1 inhibitors in urothelial cancers, non-small cell lung cancers (NSCLC) and triple negative breast cancers (TNBC)[32,33]. EMT contributes in the development ofchemoresistance in breast, pancreatic and bladder cancers[14,29,34-36]. Both adriamycin- and vinblastine-resistant MCF-7 breast cancer cells lose epithelial markers like keratin-19 and uvomorulin expression, and overexpress mesenchymal markers such as vimentin. They also show reduced desmosome formation and tight junctions[37,38]. This resistance is achieved by a variety of signalling events that contribute to EMT. Gottesman *et al.*[39] reviewed the role of ATP-binding cassette (ABC) transporters in the multi-drug resistance of cancers. These are overexpressed in many cancers and the inhibition of them contributes to the reversal of resistance. MRP1 (ABCC1) is a multidrug ABC transporter that gives resistance to lung, breast, and prostate cancers, and neuroblastoma, and is expressed by the transcription factor N-myc proto-oncogene (MYCN)[40]. Breast cancer resistance protein (BRCP/ABCG2) is one more example of an ABC transporter whose normal function removing toxins and xenobiotics in healthy tissues are repurposed in cancers towards multidrug resistance[41]. Most of these ABC transporters are overexpressed by transcription factors such as Twist, Snail, and FOXC2, which contribute to the progression of EMT[42]. Thus, targeting EMT via these transcription factors is a viable strategy to overcome drug resistance. Witta *et al.*[43] proved that the reversal of EMT by overexpressing E-cadherin successfully sensitized NSCLC towards epidermal growth factor receptor (EGFR) inhibitors. They did this by pre-treating resistant cells with MS-275, a histone deacetylase (HDAC) inhibitor which activated E-cadherin, followed by treatmentwith gefitinib and erlotinib, which are EGFR inhibitors[43]. Recent clinical work also suggests that EMT contributes to drug resistance by increasing cancer stem cell (CSC) markers. Treatment of a patient with metastatic prostate cancer with the PARP inhibitor talazoparib increased NANOG, CD133, CD44v6, and ALDH1, which are CSC markers[44]. TGF-β1-induced EMT increases ALDH expression and leads to the generation of CSCs. This contributes to decreased drug sensitivity, increased proliferation, invasion and metastasis, and poor prognosis[45]. Owing to the fact that EMT is often regulated by many signalling pathways, it is nearly impossible to target it with single-agent therapies[46]. Optimal combinatorial approaches are needed for specific types of cancer after understanding the molecular drivers of EMT.

EXISTING SCREENING TECHNOLOGIES FOR TARGETING EMT AND THEIR LIMITATIONS

Many drugs were tested and their variable efficiency against EMT has been demonstrated. These drugs encompass all the pathways which contributeto EMT development[47]. The scope of this review is the methodologies for screening drugs against EMT. They can be broadly divided into two categories: 2-dimensional (2D) models, and 3-dimensional (3D) models. Alongwith increased dimensionality, the complexity of 3D models increases compared to 2D models, and there is decreased ease of handling.

2D models

As EMT is a 3-dimensional phenomenon, it is not possible to model it in 2-dimensions. Thus, these 2D models utilize only certain EMT features for designing the assays. These are easy to handle and well amenable to high throughput screening (HTS) systems. Most of these assays are based on reporter constructs which can measure the activation of CDH1 promoter (for E-cadherin protein) elements. Chua *et al.*[48] developed a high throughput assay based on EMT spot migration, where they seeded epithelial cells as a spot and induced it with EMT inducers. The resulting mesenchymal cells will be loosely arranged and the area of the spot increases. They screened several drugs on inhibition of this spot area and found that ALK5,

MEK, and SRC inhibitors worked well as they are associated with signalling pathways that can activate EMT[48]. Huang *et al.*[49] made a cell line-based screening system for EMT inhibitors using 43 ovarian cancer cell lines. They categorized these cell lines into four subgroups, based on their levels of expression of EMT markers: epithelial, intermediate E, intermediate M, and mesenchymal types. Based on these types, the EMT inhibiting drugs can be promoters of epithelial markers in the epithelial and intermediate E groups, or inhibitors of mesenchymal markers in the other two groups. Using this approach, they identified an Src kinase inhibitor, Saracatinib (AZD0530) which reversed E-cadherin expression in the intermediate M subgroup[49]. Zhang *et al.*[50] developed a microfluid-based high throughput screening system, named mesenchymal migration chip. With this, many drugs can be screened for their inhibitory potential of mesenchymal migration, which might lead to the reversal of EMT. The migration velocity of individual cells and the total percentage of migrated cells can be quantifiedusing this assay[50].

The inhibition of E-cadherin expression is necessary for EMT progression. Using this feature, a bioluminescence-based epithelial marker promoter induction screening system was developed, whereby the promoter of E-cadherin or epithelial-specific epidermal growth factor receptor family member ERBB3was cloned in a luciferase vector. Several HDAC inhibitors were identified using this system[51]. A further high throughput study also utilized E-cadherin expression analysis with immunofluorescence in pancreatic cancer. It also identified a novel HDAC inhibitor 1-(benzylsulfonyl) indoline among 17 other compounds that worked in a dose-dependent manner. Positive hits were also validated for inhibiting tumorsphere formation[52].

All the models discussed above can test drugs for EMT inhibition only when EMT is induced by ligands such as TGF-β, epidermal growth factor (EGF), and hepatocyte growth factor. However, none of them can measure the effect of physical and mechanical forces due to tumour growth which can also induce EMT. Nakanishi *et al.*[53] recently developed a better assay for solving this problem. Here they used photoactivatable gold substrate which can change from non-cell-adhesive to cell-adhesive upon treatment with UV light. First, single irradiation with a specific pattern is performed, and cells will be seeded to confluency. Cells will grow tightly only in those irradiated regions. After the second irradiation for the remaining areas is given, cells can move into the surrounding regions, because of the mechanical force induced on the surrounding cells by the central cells. If EMT is successful, the spot size will increase and if the inhibitors were able to suppress EMT, the spot size will not increase. If the drugs can kill, then the spot size will decrease. By employing this assay, the group discovered nanaomycin H as a novel EMT inhibitor which can specifically kill EMT-induced cells[53].

3D models

Notwithstanding the advantages with 2D models, they cannot completely mimic the 3-dimensional nature of the tumour. These 2D cultures are known to induce certain cellular features, which are different from the cells grown *in vivo*. 2D cultures in polystyrene plates enhance integrin signalling, as the cells are dependent on the surface attachment for growth. Because of this, growth factors like EGF and TGF-α cannot induce further growth, but induce proliferation in 3D and *in-vivo* models[54]. Only 3D cultures can efficiently induce EMT-related transcription factors when compared with 2D cultures. This is achieved by the activation of nuclear factor-κB in 3D cultures. The EMT-induced cells were able to form successful metastases[55]. The 3D culture was first shown by Sutherland *et al.*[56] in 1971 as multi-cell spheroids and it was suggested that the growth properties of these spheroids are more similar to *in vivo* tumours. Later, in 1990, the Bjerkvig group showed the growth of multicellular organotypic spheroids to be similar to transplanted mouse tumours[57]. It was subsequently discovered that a whole cancer can be regenerated using one cell type, which is termed CSC[58]. This led to the development of tumorsphere cultures in almost all types of cancers and the development of drug screening systems for CSCs[59]. EMT plays a crucial role in the development and maintenance of CSCs. Mesenchymal traits are common for normal stem cells as well as for CSCs[60].

As the attachment of cells to the surface is too strong in 2D cultures using polystyrene plates, efforts were made to minimize the cell to surface attachment and to increase cell to cell contacts. One such effort was done by the Fujibayashi group, who designed nano-culture plates (NCP) using inorganic nanomaterial scaffolds and nanoimprinting technology. Increased cell migration and spheroid formation at different locations on the plates was demonstrated, unlike in 2D plates[61]. Arai *et al.*[62] used this NCP-based gel and soft-agar free 3D-HTS system for screening 1,330 compounds for spheroid EMT inhibitory (SEMTIN) activity. They found 9 compounds with significant activity. SB-525334, a TGF-βR1 inhibitor, and SU9516, a CDK2 inhibitor, were shown to have SEMTIN activity. This is also an example of the successful culture of A549 lung cancer cells on the NCP platform and its EMT features were established clearly. Aref *et al.*[63] developed a 3D microfluidic assay that provides a 3D microenvironment for cells to mimic EMT using HUVEC cells, and the assay is quantifiable as well. They co-cultured A549 lung cancer cells with HUVEC cells in this system and compared several metastatic inhibiting drugs between 2D and 3D models. In their system, A83-01, which is a TGF-βR inhibitor, significantly lost potency in the 3D system (5 nmol/L *vs.* 2.5 μmol/L)[63]. 3D models of HNSCC expressed EMT markers better than 2D models. They also showed a decreased sensitivity to cisplatin and cetuximab, unlike in 2D models. This suggests that 3D models can provide better simulations of drug activity[64]. These reports suggest that 3D models are far superior to 2D models in mimicking EMT phenomena and predicting the potency of the drugs.

NEXT-GENERATION DISCOVERY ATTEMPTS FOR CONTROLLING EMT

Though 3D assays are better than the 2D assays for efficient drug screening, they are limited by the ease of the experiment analysis for doing primary and secondary assays. Most of the 3D assays utilize artificial substrates or gel components, which in turn make those assays different from *in vivo* or clinical models. Although they can form 3D architects, they might be different from the original ones and might have been induced by the nature of the materials that were used. Tumorspheresare also no longer considered as exact replicas of the original tumour. They are only rich in the CSC population and lack the heterogeneity of the tumour *in vivo*[65]. Maintenance of tumorspheres for long term with the same properties and composition is also challenging[66].

Owing to all these problems with traditional 3D-HTS systems, organoid cultures are becoming more popular, as they are small pieces of original patient-derived tumours that were grown in laboratory conditions. They form a full tumour and express the markers and mimic the organ properties from which they were derived[67]. Organoids use basement membrane components like Matrigel, which arecloser to the natural system compared to ultralow attachment surfaces for tumorspheres which cannot be found in nature[68]. Though the term organoid has been used in many different contexts, its actual popularity started when intestinal organoids were developed by the Hans Clevers group, using $Lgr5^+$ stem cells[69]. LGR5 was found to activate EMT in glioma stem cells and is a better therapeutic target for EMT control. It functions through the WNT/β-catenin pathway[70]. Because of the importance of organoids in many fields, it was considered as the "Method of 2017"[71]. Fan *et al.*[72] extensively reviewed the organoid models that were developed for different types of cancers.

Patient-derived tumour organoids are cheaper, faster, and easier to handle compared to patient-derived tumour xenografts, which were traditionally used. These can also be used for high throughput screening of drugs, that can be administered immediately to the corresponding patients[72,73] (personalised cancer medicine). Despite the current lack of studies reporting on EMT using organoids, many more are expected soon. Hypoxic gradients that are much needed for EMT induction cannot be modelled by traditional 3D assays but can be done by using organoid models[74]. Hahn *et al.*[75] developedan intestinal organoid-based EMT model by inducing intestinal organoids with TGF-β1 and TNF-α. This suggests that it is possible to get EMT models for different types of cancers, which can be further used for high throughput drug screening. Drugs that can come through the organoid screen are more likely to work better in patients and

it helps in improving personalised medicine[73,76]. However, there are two main disadvantages of organoid models: (1) cost; and (2) better and easier assays need to be developed further for efficient screening. As these organoids harbourthe heterogeneity of the tumour, classical assays based on a single type of cells are not enough. This research area is mainly dependent on omics studies, such as genomics, transcriptomics, and proteomics[73,77]. Extensive research in this novel field might contribute to overcoming these limitations.

CONCLUSION

Decades of research and accumulating literature has revealed the complexity of EMT driven by its intricate network of physiology that is difficult to imitate under lab conditions as experimental model systems. Though many models are being used, they have their limitations and are currently unable to mimic the *in vivo* nature of EMT sufficiently. Researchers still struggle with falsepositive and false negative results due to these problems. Therefore, the problem should be addressed with a holistic approach including a combination of feasible 2D, 3D and organoid models along with omics studies to achieve meaningful outcomes for patient care. Given the modern progression of science and technology, with the merger of advanced interdisciplinary tools, we may soon see game-changing giant leaps of success in the precision targeting of EMT.

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Authors' contributions

Wrote the manuscript with input received from Irlapati V and Dravida S: Mokhamatam RB
Contributed in supporting with references, summarizing the interpretation with critical feedback: Irlapati VK
Conceived the framework of the review, supported by providing overall direction with the flow, edited the manuscript: Dravida S

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Conflict of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

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REFERENCES

1. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat Rev Clin Oncol 2017;14:611-29.
2. Kim YS, Yi BR, Kim NH, Choi KC. Role of the epithelial-mesenchymal transition and its effects on embryonic stem cells. Exp Mol Med 2014;46:e108.
3. Larue L, Bellacosa A. Epithelial-mesenchymal transition in development and cancer: role of phosphatidylinositol 3' kinase/AKT pathways. Oncogene 2005;24:7443-54.
4. Nieto MA, Huang RY, Jackson RA, Thiery JP. EMT: 2016. Cell 2016;166:21-45.
5. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer 2006;6:392-401.
6. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442-54.
7. Boyer B, Vallés AM, Edme N. Induction and regulation of epithelial-mesenchymal transitions. Biochem Pharmacol 2000;60:1091-9.
8. Yeaman C, Grindstaff K, Hansen M, Nelson W. Cell polarity: versatile scaffolds keep things in place. Curr Biol 1999;9:R515-7.
9. Nieto MA. The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol 2002;3:155-66.
10. Huang RY, Guilford P, Thiery JP. Early events in cell adhesion and polarity during epithelial-mesenchymal transition. J Cell Sci 2012;125:4417-22.
11. Boyer B, Thiery JP. Epithelium-mesenchyme interconversion as example of epithelial plasticity. APMIS 1993;101:257-68.
12. Hay ED. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel) 1995;154:8-20.
13. Brabletz T, Lyden D, Steeg PS, Werb Z. Roadblocks to translational advances on metastasis research. Nat Med 2013;19:1104-9.
14. Fischer KR, Durrans A, Lee S, Sheng J, Li F, et al. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature 2015;527:472-6.
15. Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol 2000;2:76-83.
16. Batlle E, Sancho E, Francí C, Domínguez D, Monfar M, et al. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat Cell Biol 2000;2:84-9.
17. Peinado H, Portillo F, Cano A. Transcriptional regulation of cadherins during development and carcinogenesis. Int J Dev Biol 2004;48:365-75.
18. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 2004;117:927-39.
19. Nawshad A, Hay ED. TGFbeta3 signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. J Cell Biol 2003;163:1291-301.
20. Aiello NM, Brabletz T, Kang Y, Nieto MA, Weinberg RA, et al. Upholding a role for EMT in pancreatic cancer metastasis. Nature 2017;547:E7-8.
21. Ye X, Brabletz T, Kang Y, Longmore GD, Nieto MA, et al. Upholding a role for EMT in breast cancer metastasis. Nature 2017;547:E1-3.
22. Xu Q, Zhang Q, Ishida Y, Hajjar S, Tang X, et al. EGF induces epithelial-mesenchymal transition and cancer stem-like cell properties in human oral cancer cells via promoting Warburg effect. Oncotarget 2017;8:9557-71.
23. Katsuno Y, Lamouille S, Derynck R. TGF-β signaling and epithelial-mesenchymal transition in cancer progression. Curr Opin Oncol 2013;25:76-84.
24. Hu W, Wang Z, Zhang S, Lu X, Wu J, et al. IQGAP1 promotes pancreatic cancer progression and epithelial-mesenchymal transition (EMT) through Wnt/β-catenin signaling. Sci Rep 2019;9:7539.
25. Wang Z, Li Y, Kong D, Sarkar FH. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. Curr Drug Targets 2010;11:745-51.
26. Wang F, Ma L, Zhang Z, Liu X, Gao H, et al. Hedgehog Signaling Regulates Epithelial-Mesenchymal Transition in Pancreatic Cancer Stem-Like Cells. J Cancer 2016;7:408-17.
27. Gonzalez DM, Medici D. Signaling mechanisms of the epithelial-mesenchymal transition. Sci Signal 2014;7:re8.
28. Al Ameri W, Ahmed I, Al-Dasim FM, Ali Mohamoud Y, Al-Azwani IK, et al. Cell type-specific TGF-β mediated EMT in 3D and 2D models and its reversal by TGF-β receptor kinase inhibitor in ovarian cancer cell lines. Int J Mol Sci 2019;20:3568.
29. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules 2016;21:965.
30. Akalay I, Janji B, Hasmim M, Noman MZ, André F, et al. Epithelial-to-mesenchymal transition and autophagy induction in breast carcinoma promote escape from T-cell-mediated lysis. Cancer Res 2013;73:2418-27.
31. Noman MZ, Janji B, Abdou A, Hasmim M, Terry S, et al. The immune checkpoint ligand PD-L1 is upregulated in EMT-activated human breast cancer cells by a mechanism involving ZEB-1 and miR-200. Oncoimmunology 2017;6:e1263412.
32. Wang L, Saci A, Szabo PM, Chasalow SD, Castillo-Martin M, et al. EMT- and stroma-related gene expression and resistance to PD-1 blockade in urothelial cancer. Nat Commun 2018;9:3503.
33. Soundararajan R, Fradette JJ, Konen JM, Moulder S, Zhang X, et al. Targeting the interplay between epithelial-to-mesenchymal-transition and the immune system for effective immunotherapy. Cancers (Basel) 2019;11:714.
34. Arumugam T, Ramachandran V, Fournier KF, Wang H, Marquis L, et al. Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. Cancer Res 2009;69:5820-8.
35. McConkey DJ, Choi W, Marquis L, Martin F, Williams MB, et al. Role of epithelial-to-mesenchymal transition (EMT) in drug sensitivity and metastasis in bladder cancer. Cancer Metastasis Rev 2009;28:335-44.
36. Zheng X, Carstens JL, Kim J, Scheible M, Kaye J, et al. Epithelial-to-mesenchymal transition is dispensable for metastasis but induces

chemoresistance in pancreatic cancer. Nature 2015;527:525-30.
37. Sommers CL, Heckford SE, Skerker JM, Worland P, Torri JA, et al. Loss of epithelial markers and acquisition of vimentin expression in adriamycin- and vinblastine-resistant human breast cancer cell lines. Cancer Res 1992;52:5190-7.
38. Huang J, Li H, Ren G. Epithelial-mesenchymal transition and drug resistance in breast cancer (Review). Int J Oncol 2015;47:840-8.
39. Gottesman MM, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2002;2:48-58.
40. Munoz M, Henderson M, Haber M, Norris M. Role of the MRP1/ABCC1 multidrug transporter protein in cancer. IUBMB Life 2007;59:752-7.
41. Natarajan K, Xie Y, Baer MR, Ross DD. Role of breast cancer resistance protein (BCRP/ABCG2) in cancer drug resistance. Biochem Pharmacol 2012;83:1084-103.
42. Saxena M, Stephens MA, Pathak H, Rangarajan A. Transcription factors that mediate epithelial-mesenchymal transition lead to multidrug resistance by upregulating ABC transporters. Cell Death Dis 2011;2:e179.
43. Witta SE, Gemmill RM, Hirsch FR, Coldren CD, Hedman K, et al. Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. Cancer Res 2006;66:944-50.
44. Navas T, Kinders RJ, Lawrence SM, Ferry-Galow KV, Borgel S, et al. Clinical evolution of epithelial-mesenchymal transition in human carcinomas. Cancer Res 2020;80:304-18.
45. Shuang ZY, Wu WC, Xu J, Lin G, Liu YC, et al. Transforming growth factor-β1-induced epithelial-mesenchymal transition generates ALDH-positive cells with stem cell properties in cholangiocarcinoma. Cancer Lett 2014;354:320-8.
46. Barneh F, Mirzaie M, Nickchi P, Tan TZ, Thiery JP, et al. A rational drug combination design to inhibit epithelial-mesenchymal transition in a three-dimensional microenvironment. bioRxiv 2017;148767.
47. Marcucci F, Stassi G, De Maria R. Epithelial-mesenchymal transition: a new target in anticancer drug discovery. Nat Rev Drug Discov 2016;15:311-25.
48. Chua KN, Sim WJ, Racine V, Lee SY, Goh BC, et al. A cell-based small molecule screening method for identifying inhibitors of epithelial-mesenchymal transition in carcinoma. PLoS One 2012;7:e33183.
49. Huang RY, Wong MK, Tan TZ, Kuay KT, Ng AH, et al. An EMT spectrum defines an anoikis-resistant and spheroidogenic intermediate mesenchymal state that is sensitive to e-cadherin restoration by a src-kinase inhibitor, saracatinib (AZD0530). Cell Death Dis 2013;4:e915.
50. Zhang Y, Zhang W, Qin L. Mesenchymal-mode migration assay and antimetastatic drug screening with high-throughput microfluidic channel networks. Angew Chem Int Ed Engl 2014;53:2344-8.
51. Tang HM, Kuay KT, Koh PF, Asad M, Tan TZ, et al. An epithelial marker promoter induction screen identifies histone deacetylase inhibitors to restore epithelial differentiation and abolishes anchorage independence growth in cancers. Cell Death Discov 2016;2:16041.
52. Polireddy K, Dong R, McDonald PR, Wang T, Luke B, et al. Targeting epithelial-mesenchymal transition for identification of inhibitors for pancreatic cancer cell invasion and tumor spheres formation. PLoS One 2016;11:e0164811.
53. Nakanishi J, Sugiyama K, Matsuo H, Takahashi Y, Omura S, et al. An application of photoactivatable substrate for the evaluation of epithelial-mesenchymal transition inhibitors. Anal Sci 2019;35:65-9.
54. Mizushima H, Wang X, Miyamoto S, Mekada E. Integrin signal masks growth-promotion activity of HB-EGF in monolayer cell cultures. J Cell Sci 2009;122:4277-86.
55. Kumar M, Allison DF, Baranova NN, Wamsley JJ, Katz AJ, et al. NF-κB regulates mesenchymal transition for the induction of non-small cell lung cancer initiating cells. PLoS One 2013;8:e68597.
56. Sutherland RM, McCredie JA, Inch WR. Growth of multicell spheroids in tissue culture as a model of nodular carcinomas. J Natl Cancer Inst 1971;46:113-20.
57. Bjerkvig R, Tønnesen A, Laerum OD, Backlund EO. Multicellular tumor spheroids from human gliomas maintained in organ culture. J Neurosurg 1990;72:463-75.
58. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730-7.
59. Lee CH, Yu CC, Wang BY, Chang WW. Tumorsphere as an effective in vitro platform for screening anti-cancer stem cell drugs. Oncotarget 2016;7:1215-26.
60. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, et al. Epithelial-mesenchymal transition generates cells which have stem cell properties. Cell 2009;133:704-15.
61. Yoshii Y, Waki A, Yoshida K, Kakezuka A, Kobayashi M, et al. The use of nanoimprinted scaffolds as 3D culture models to facilitate spontaneous tumor cell migration and well-regulated spheroid formation. Biomaterials 2011;32:6052-8.
62. Arai K, Eguchi T, Rahman MM, Sakamoto R, Masuda N, et al. A novel high-throughput 3D screening system for EMT inhibitors: a pilot screening discovered the EMT inhibitory activity of CDK2 inhibitor SU9516. PLoS One 2016;11:e0162394.
63. Aref AR, Huang RY, Yu W, Chua KN, Sun W, et al. Screening therapeutic EMT blocking agents in a three-dimensional microenvironment. Integr Biol (Camb) 2013;5:381-9.
64. Melissaridou S, Wiechec E, Magan M, Jain MV, Chung MK, et al. The effect of 2D and 3D cell cultures on treatment response, EMT profile and stem cell features in head and neck cancer. Cancer Cell Int 2019;19:16.
65. Weiswald LB, Bellet D, Dangles-Marie V. Spherical cancer models in tumor biology. Neoplasia 2015;17:1-15.
66. Zweigerdt R, Olmer R, Singh H, Haverich A, Martin U. Scalable expansion of human pluripotent stem cells in suspension culture. Nat Protoc 2011;6:689-700.
67. Neal JT, Kuo CJ. Organoids as models for neoplastic transformation. Annu Rev Pathol 2016;11:199-220.

68. Nakamura T, Sato T. Advancing intestinal organoid technology toward regenerative medicine. Cell Mol Gastroenterol Hepatol 2018;5:51-60.
69. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 2009;459:262-5.
70. Zhang J, Cai H, Sun L, Zhan P, Chen M, et al. LGR5, a novel functional glioma stem cell marker, promotes EMT by activating the Wnt/β-catenin pathway and predicts poor survival of glioma patients. J Exp Clin Cancer Res 2018;37:225.
71. Method of the Year 2017: Organoids. Nat Methods 2018;15:1.
72. Fan H, Demirci U, Chen P. Emerging organoid models: leaping forward in cancer research. J Hematol Oncol 2019;12:142.
73. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell 2015;161:933-45.
74. Hubert CG, Rivera M, Spangler LC, Wu Q, Mack SC, et al. A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. Cancer Res 2016;76:2465-77.
75. Hahn S, Nam MO, Noh JH, Lee DH, Han HW, et al. Organoid-based epithelial to mesenchymal transition (OEMT) model: from an intestinal fibrosis perspective. Sci Rep 2017;7:2435.
76. Clevers HC. Organoids: avatars for personalized medicine. Keio J Med 2019;68:95.
77. Pauli C, Hopkins BD, Prandi D, Shaw R, Fedrizzi T, et al. Personalized in vitro and in vivo cancer models to guide precision medicine. Cancer Discov 2017;7:462-77.