

Mutant p53 hinges between epithelial-mesenchymal transition and cancer stem cells

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Increasing body of evidence ascribes tumorigenesis to the emergence of cancer stem cells (CSCs). The two main theories predicting the origin of CSCs are either transformation of adult stem cells, or de-differentiation of mature cells that is accompanied by the induction of the epithelial to mesenchymal transition (EMT) program [1]. Yet, both are associated with the accumulation of genetic and epigenetic aberrations that underlies cell plasticity [2]. Since accumulating data link mutations in the tumor-suppressor p53 with both CSCs formation and cancer-associated EMT, it is tempting to hypothesize that mutant p53 might facilitate CSCs formation by inducing EMT and cell plasticity. Here, we will evaluate the latter hypothesis by analyzing recent publications, and supporting it by our new data pertaining prostate cancer.

EMT- induced cell plasticity is associated with CSCs features

A recent accepted concept explaining cancer development and relapse is the hierarchical model, which predicts that a small subset of cells within tumors has the ability to both self-renew and to differentiate in various cell types, thus maintaining the ongoing heterogeneous neoplasm [3]. EMT that was first described as critical process in embryogenesis, was shown to be activated in various tumor cells by the EMT- transcription factors, mainly of the SLUG/SNAIL, TWIST and ZEB families. This activation was suggested to yield cells migration and metastasis. Recent studies, however, ascribe EMT, also to enhanced stemness capacity; accordingly, EMT was suggested to be a critical process in mediating CSC phenotype [1,4]. This is mainly due to the cellular changes mediated by the EMT process that is known to induce cancer cells plasticity [5]. Indeed, EMT was found significantly associated with CSCs. For example, in a mouse model for skin cancer, tumor cells that underwent EMT (Epcam⁻ cells) contained higher frequency of tumor propagating cells than that observed in the epithelial cells (Epcam⁺ cells), implying on higher stemness capacity [6]. Moreover, it was shown that induction of EMT in breast tumor-derived cell lines is associated with a higher capacity to generate mammospheres with frequent CSCs markers, thus supporting the notion that the two are interconnected [7-10]. Several experimental settings have shown that expression of the EMT transcription factors is associated with CSCs. For example, Snail was suggested to be responsible for the transition from asymmetrical to symmetrical division of colorectal CSCs by maintaining high Wnt activity, and thus propagating the CSCs pool [11]. Ectopic expression of TWIST1 in esophageal squamous cell carcinoma caused significant upregulation of OCT4, a main self-renewal mediating factor [12]. Other cancer models for Leukemia [13] and ovarian cancer [14] associated Snail and Twist expression to the CSCs marker CD44. Finally, in pancreatic cancer

mouse model, where K-Ras and p53 are mutated (KPC model), Zeb1 was found crucial for EMT, tumorigenic capacity as well as stemness phenotype [15]. Altogether, these evidence strongly link CSCs to EMT, and further suggest that EMT induction may lead to cancer stemness.

Mutant p53 mediates EMT

In a step-wise prostate transformation model we have previously shown that mutant p53 facilitates the expression of EMT related genes, including Twist1 induction and E-cadherin repression. This was accompanied with higher invasion capabilities and morphologically disrupted spheroids [16]. While Wang Z et al. showed that silencing wild-type p53 lead to EMT, migration and metastasis of hepatocellular carcinoma (HCC) cells [17], we further suggest a gain of function activities of mutant p53, that enhances this phenotype to higher extent than p53 inactivation alone [16]. The repression of E-cadherin expression by mutant p53 was also demonstrated in human colon carcinoma cell line model, HCT116. However, in this model, p53 temperature sensitive mutant p53-A143 did not show enhanced invasion [18]. The differences in the observed phenotype can stem from the p53 mutation type or cell types tested. In esophageal cells model, it was suggested that mutant p53^{R175H} cooperates with epidermal growth factor receptor (EGFR) to enhance EMT phenotype upon treatment with EMT inducer, TGFβ. This was demonstrated by lower levels of E-cadherin and higher levels of N-cadherin, Zeb1/2 and Snail, and was associated with inactivation of EGFR-induced senescence, suggesting additional role for mutant p53 and EMT in senescence checkpoint during tumorigenesis [19]. More recently, mutant p53 expressing WAP-T transgenic mice, showed higher expression of an EMT gene signature which was associated with higher tumorigenic grading, associated with enhanced vascularization and metastatic potential [20]. Several studies aimed at understanding the molecular mechanism underlying mutant p53-dependent induction of EMT, suggest that mutant p53 gain of function activities promote EMT by modulating the expression of the key EMT transcription factors. For example, our previous study attributed the induction in Twist1 levels to deregulation of epigenetic mechanisms by mutant p53. Inhibition of BMI-1 protein by mutant p53 led to the reduction in H3K27me3 on Twist1 promoter, and activation of Twist1 expression [16]. In addition, in colorectal cancer cell lines, the induced expression of the EMT transcription

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Received: June 03, 2018; **Accepted:** June 20, 2018; **Published:** June 23, 2018

factors- Snail and Slug was attributed to the mutant p53-dependent transcriptional upregulation of EFNB2 in response to DNA damage [21]. Other study suggested that in endometrial cancer, mutant p53 represses the expression of miR-130b, a negative regulator of ZEB1, and by that mediates ZEB1 elevation, leading to EMT phenotype [22].

Mutant p53 expression is correlated with CSCs features

It was recently claimed that tissues containing an increased frequency of highly dividing stem cells permit the increased incidence of mutations, and thus are prone to develop tumors [23,24]. As p53 serves as an important guardian of the genome, it is expected that it will play a major role in ensuring the quality and stability of stem cells genome [25]. Thus, it is not surprising that mutations in p53 may in turn lead to the formation of aberrant stem cells, referred as CSCs. Indeed, mutant p53 knock-in mice models have shown that bone marrow-derived hematopoietic and mesenchymal stem cells (MSCs) populations exhibit an augmented frequency of CSCs that are capable of inducing tumors *in vivo* [26,27]. This notion is further substantiated by our previous observation that mutant p53 facilitates the *in vitro* reprogramming of mouse embryonic fibroblast (MEFs) towards the generation of induced pluripotent stem cells (iPSC). This facilitated process lead to the generation of malignant iPSC that in that respect can be regarded as CSCs [28]. These data emphasize the role of mutant p53 in mediating harmful de-differentiation, and by that accelerating cancer stem cells malignancy. Accordingly, recent evidence suggest that in various cancer types the expression of mutant p53 is correlated with CSCs features. For example, p53 protein derived of a deletion mutation that disrupts p53 DNA binding domain, was found accumulated in multipotent stem cells of brain sub-ventricular zone and in transit-amplifying progenitors, which are suggested to be the cellular origin of glioma that accounts for the development of glioblastoma [29]. In other cancer models mutant p53 was associated with enhanced CSCs features. For example, mutant p53^{R248W/P72R} in human osteosarcoma cells promoted CSCs properties such as formation of larger sarco-spheres and enhanced expression of stemness genes (e.g. CD133, ABCG2, Nanog) [30]. Accordingly, in glial and breast-derived cancer cells, mutant p53 expression was found correlated with enhanced spheres

formation and induction of the stemness markers CD133 and CD44 [31]. Additionally, we and others showed that colorectal cancer cells expressing mutant p53 are associated with larger sub-population of cells expressing CSCs markers and drug resistance [32, 33]. Interestingly, recently, we observed that mutant p53 expressing MSCs-derived highly aggressive tumor cells, express an embryonic gene signature that may testify that such CSCs underwent re-programing [27]. Altogether, these data further support the role for mutant p53 gain of function in facilitating the oncogenic characteristics of CSCs.

Mutant p53 plays a role in facilitating CSCs features of prostate tumor cells in association with inducing the EMT process

When focusing on better understanding mutant p53 gain of function activities in promoting cancer development, we recently suggested that mutant p53 is associated with higher expression of CSCs markers in colorectal cancer [33]. Moreover, in a prostate transformation model, epithelial prostate cells were immortalized (EP156T) and introduced with either mutant p53^{R175H} or inactivation of p53 by the dominant negative p53 peptide GSE56, to study p53 role in prostate carcinogenesis. Results obtained indicated that mutant p53 induced EMT in a gain of function manner [16]. As induction of the EMT program was suggested to be associated with CSCs, we hypothesized that mutant p53 augmented the oncogenic CSCs features through EMT. Analysis of the expression of CD44^{high}/CD24^{low} CSCs markers [34] in EP156T prostate indicated that in mutant p53^{R175H} expressing cells the CD44^{high}/CD24^{low} sub-population is larger compared with wild-type p53 and GSE56 expressing cells. This observation was further corroborated in additional prostate cancer cell line system, DU145, which endogenously expresses mutant p53^{P223L,V274F} [16], confirming the conclusion that prostate cancer cell-lines, expressing mutant p53, harbor larger CD44^{high}/CD24^{low} sub-population (Figure 1). These observations suggest that mutant p53 gain of function is associated with an increased tumorigenic CSCs sub-population. In accordance with our observation that EP156T and DU145 cells expressing mutant p53 are associated with higher EMT features [16], we found that while the CSCs sub-population was enriched for EMT transcription factors

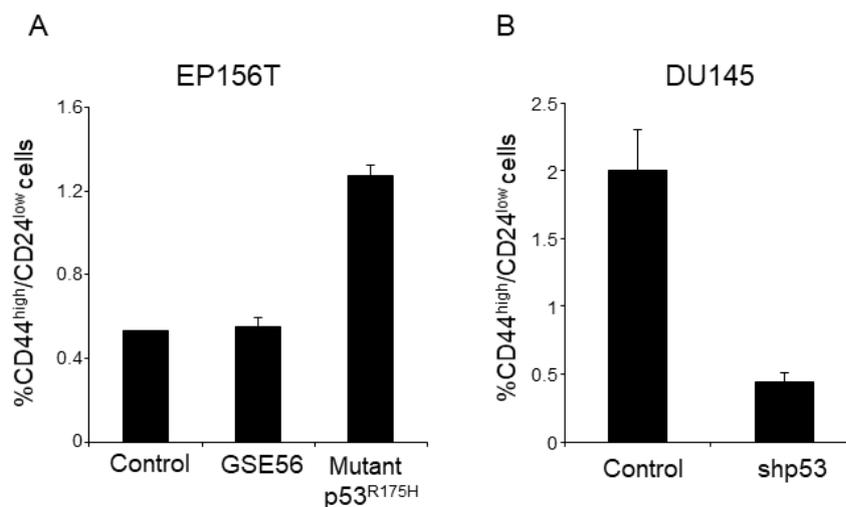


Figure 1. Mutant p53 expressing cells contain larger CD44^{high}/CD24^{low} sub-population. The EP156T (A) and the DU145 (B) cells were established and maintained as described previously [16]. Briefly, epithelial prostate cells were immortalized (EP156T) and introduced with either mutant p53^{R175H} or inactivation of p53 by the dominant negative p53 peptide GSE56, or control vector. The DU145 cell line, endogenously expressing mutant p53^{P223L,V274F}, were stably introduced with shRNA against p53 to knock-down mutant p53^{P223L,V274F} expression. The established cells were immunostained with anti-CD44-APC conjugated (eBioscience) and anti-CD24-PE conjugated (BD Bioscience) antibodies followed by evaluation of the size of CD44^{high}/CD24^{low} sub-population by FACS analysis. FACS procedure was performed as described previously [29]. Graph presenting an average of three experiments.

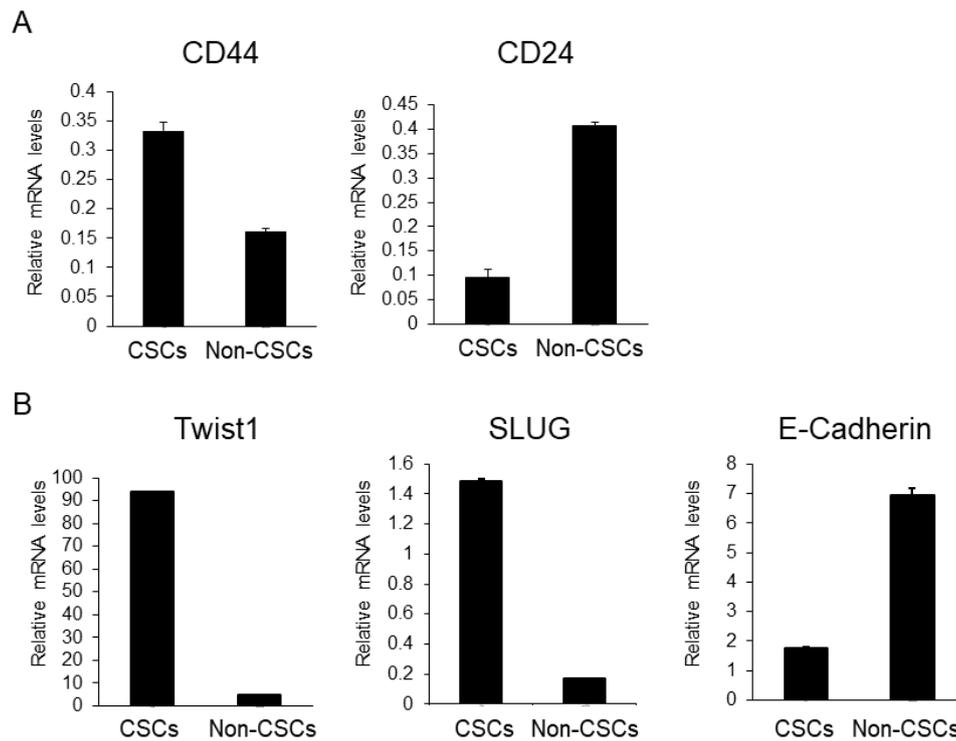


Figure 2. CD44^{high}/CD24^{low} sub-population is associated with EMT features in mutant p53^{P223L-V274F} expressing DU145 cells. The DU145 cells were immunostained with anti-CD44 and anti-CD24 antibodies (as performed in Figure 1), and the CD44^{high}/CD24^{low} sub-population was sorted out of the whole DU145 cell population by FACS. (A) To ensure sorting efficiency, CD44 and CD24 mRNA expression levels were measured in the CD44^{high}/CD24^{low} (CSCs) sub-population, in comparison to the CD44^{low}/CD24^{high} (non-CSCs) sub-population, by quantitative real time PCR (qRT-PCR). (B) mRNA levels of EMT transcription factors, Twist and SLUG, and the expression of the epithelial marker, E-Cadherin, were measured by qRT-PCR in the CSCs sub-population, and in the non-CSCs sub-population as a control. FACS sorting procedure and qRT-PCR was conducted as previously described [29]. Graph presenting an average of three experiments.

Twist and SLUG, the E-Cadherin expression was decreased (Figure 2), thus inferring that the cellular sub-population that gained CSCs features was most likely dictated by EMT.

As these observations are mutant p53 dependent, it is tempting to speculate that mutant p53 mediates CSCs features of cancer cells, via induction of EMT program. This hypothesis is supported by a recently published paper suggesting that mutant p53 facilitates CSCs features of glioblastoma and breast cancer cells by activating YAP/TAZ signaling [31] that was already proposed as regulator of EMT process [35]. Once established, this important hinge between EMT and CSCs formation in a mutant p53 dependent manner, may be regarded as a milestone in the understanding of the molecular events underlying the augmented oncogenic aggressiveness of CSCs.

Acknowledgements

Research in the laboratory of Varda Rotter is supported by a Center of Excellence grant from Flight Attendant Medical Research Institute (FAMRI) and Israel Science Foundation ISF-MOKED. V.R. is the incumbent of the Norman and Helen Asher Professorial Chair for Cancer Research at the Weizmann Institute.

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