List of ten best papers of the candidate (not to exceed 3000 words):

The following are the 10 best papers of the candidate contributing to the area of **"Epithelial to Mesenchymal Transition (EMT)"** for which the present nomination has been made.

No	Names of the author(s)	Year	Title of the paper	Name of journal	Volume and page	Impact Factor	Citations (Scopus)
1.	Bapat SA, Mali AM, Koppikar CB, Kurrey NK.	2005	Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer.	Cancer Research	65:3025-3029.	8.619	582
2.	Kurrey NK, Kumar A, Bapat SA.	2005	Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level.	Gynecologic Oncology	97:155-65.	2.919	228
3.	Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, Bapat SA.	2009	Snail and Slug mediate radio- and chemo-resistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells.	Stem Cells	27(9): 2059-2068.	5.614	504
5.	Gardi NL, Deshpande TU, Kamble SC, Budhe SR, Bapat SA.	2014	Discrete molecular classes of ovarian cancer suggestive of unique mechanisms of transformation and metastases.	Clinical Cancer Research	20:87-99.	8.911	26
4.	Khirade MF, Lal G, Bapat SA.	2015	Derivation of a fifteen gene prognostic panel for six cancers	Scientific Reports	5:13248	4.120	20
6.	Kumar B, Uppuladinne MVN, Jani V, Sonavane U, Joshi RR, Bapat SA.	2015	Auto-regulation of SNAI2 mediates its activity during epithelial to mesenchymal transition.	Biochimica et Biophysica Acta (BBA) - Gene Reg. Mech.	1849:1209-1219	5.18	9
7.	Varankar SS, Bapat SA.	2018	Migratory Metrics of Wound Healing: A Quantification Approach for *in vitro* Scratch Assays.	Front Oncol.	8: 633	4.137	14
8.	Varankar SS, More M, Abraham A, Pansare K, Kumar B, Narayanan NJ, Jolly MK, Mali AM, Bapat SA.	2020	Functional balance between Tcf21-Slug defines cellular plasticity and migratory modalities in high grade serous ovarian cancer cell lines	Carcinogenesis	41:515-526	5.072	8
9.	Kamble SC, Sen A, Dhake RD, Joshi AN, Midha D, Bapat SA.	2019	Clinical Stratification of High-Grade Ovarian Serous Carcinoma Using a Panel of Six Biomarkers	J Clin Med.	8: E330	5.583	3
10.	Kalra RS, Soman GS, Parab PB, Mali AM, Varankar SS, Naik RR, Kamble SC, Dhanjal JK, Bapat SA.	2022	A Monoclonal Antibody against Annexin A2 targets stem and progenitor cell fractions in tumors.	Translational Oncology	15(1):101257.		1

Highlighting the important discoveries/contributions described in them briefly –

1. *Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Research. 2005; 65:3025-9.*

   This was the first global report of the putative identification and isolation of cancer stem cells (CSCs) in ovarian cancer. The germ of the idea that epithelial to mesenchymal transition (EMT) may be associated with CSCs was seeded during the data generated in this study when it was observed that the EMT- associated transcription factors Snail and Slug were expressed in clones associated with 'stem cell-like' features. Such an observation was not documented at the time and was completely novel. Even today the nominee receives several national and international invitations to deliver lectures in the field of CSCs and EMT. This research report is highly cited (nearing 600 citations).

2. *Kurrey NK, K A, Bapat SA. Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. Gynecol Oncol. 2005; 97:155-65.*

   We further wished to identify the role that these two EMT-TFs (Snail and Slug) played in ovarian cancer resulting in downregulation of the cytoskeletal component Cytokeratin 18 and upregulation of Vimentin, and correlating with enhanced cell migration - invasion, *in vitro* clonogenecity, along with *in vivo* tumorigenicity and metastases. At a mechanistic level, we demonstrated that Snail mediates transcriptional repression of components of adherens and tight junctions, while Slug additionally represses components of gap junctions. This concertedly dissolves the intercellular adhesion. Further activation of these EMT-TFs under hypoxic conditions revealed a rapid upregulation of Slug as an immediate reaction that probably triggers off a signaling cascade leading to Snail expression. This suggested that Slug and Snail may have common as well as distinct roles in ensuring tumor cell survival. The study, being one of the first to resolve the molecular mechanisms of EMT in cancer is highly cited (>200 citations).

3. *Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, Bapat SA. Snail and Slug mediate radio- and chemo-resistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. Stem Cells. 2009 27(9):2059-2068.*

   Towards a deeper understanding of the mechanistic implications of the 2 EMT-TFs in cancer, we compared their genome-wide transcirptional targets in steady-state tumor cell growth and under conditions of stress. This exposed that these EMT-TFs, besides driving cell migration also 'de-repress' self-renewal molecules including Nanog and *KLF4*, and support acquisition of resistance to p53-mediated apoptosis that together led to impose a 'stem-like' state to tumor cells under specific conditions of stress. This was one of the first reports that provided a mechanistic understanding of CSC enrichment following chemotherapy and is considered an important milestone in the field. Our findings complemented a study from Prof. Robert Weinberg's lab at MIT (2008) that implied another EMT-TF *viz.* Twist in the dedifferentiation of tumor cells to stem cells. Besides receiving over 500 citations, this study was the first of a series of studies in the nominee's lab that integrated computation of 'big data' with experimental validation (and one of the first from India to do so in cancer biology).

4. *Gardi NL, Deshpande TU, Kamble SC, Budhe SR, Bapat SA. Discrete molecular classes of ovarian cancer suggestive of unique mechanisms of transformation and metastases. Clinical Cancer Research, 2014,20:87-99.*

Our confidence in the strength and validity of computation-directed findings further drove us to develop systems analyses of epigenetic, expression and proteomic signatures within tumors of a cohort of ovarian cancer patients that represent heterogeneous cell compositions and molecular expression patterns. This led to the resolution of 3 discrete molecular tumor classes in high-grade serous ovarian cancer (HGSC), each with unique regulatory networks, pathways and modes of metastases. Notably, a defining feature of one of these classes was Slug-driven EMT, while a second class presented a strong epithelial identity that resisted EMT. These findings published in **Clinical Cancer Research** in 2014 provided several leads at the basic and translational level that were explored further and reported as seem in the publications listed below.

5. *Khirade MF, Lal G, Bapat SA., Derivation of a fifteen gene prognostic panel for six cancers., Scientific Reports. 5, 2015; pp: 13248.*

   In helping other research groups in India understand their expression data, the nominee recognized exclusive "Epithelial" and "EMT" molecular patterns in expression datasets across several other tumor types. To formalize these observations, she performed a pan-cancer analysis of gene expression datasets in eleven cancer types and resolved conserved modules of highly correlating EMT-associated genes and interactive, regulatory networks in glioblastoma, breast, ovary, colon, rectal and lung cancers, which in turn defined functional pathways of metastases, cell migration, metastases, oncogenic transformation, resistance to apoptosis and senescence and immune regulation. PRRX1, AIF1 and Slug were suggested to be master regulators governing these modules. Survial and correlation analyses within these cohorts further led to development of a 'GBOCRL-IIPr' panel of 15 risk genes that potentially predict patient outcomes in these six cancers.

6. *Kumar B, Uppuladinne MVN, Jani V, Sonavane U, Joshi RR, Bapat SA. Auto-regulation of SNAI2 mediates its activity during epithelial to mesenchymal transition. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 1849, 2015; 1209-1219.*

   The nominee then undertook a deeper biochemical study of Slug, a five C2H2 zinc finger (ZF) motif transcription factor that recognizes and binds to E-box (CACC/GGTG) consensus elements in the promoters of its target genes to achieve transcriptional repression. This revealed that Slug directly activates its own expression by preferential binding to specific E-box elements in the distal binding region of its promoter under conditions of stress. The detailed approach identified that while the first ZF does not contribute to the transcription-associated functions of Slug, the remaining four ZFs are involved in regulating the expression of target genes with ZF3 and ZF4 being more crucial than ZF2 or ZF5. Moreover, recognition by and binding preferences of ZFs are defined through intrinsic differences in the E-box core base pairs and/or flanking sequences, with the S2 E-box element being most crucial for autoregulation. Importantly, specific target E-box recognition and binding are defined by the cellular context, implying that *in silico* and/or biochemical preferences may not always accurately predict *in situ* events. This study constitutes a novel understanding of transcriptional regulation of Slug.

7. *Kamble SC, Sen A, Dhake RD, Joshi AN, Midha D, Bapat SA. Clinical Stratification of High-Grade Ovarian Serous Carcinoma Using a Panel of Six Biomarkers. J Clin Med. 2019 Mar 8;8(3). pii: E330. doi: 10.3390/jcm8030330.*

   The clinical relevance of HGSC molecular stratification was explored through collaboration with well-established pathologists from AFMC, KEM and Inlaks-Budhrani Hospital (Pune) and TMC (Kolkata). This involved extensive development of standard operating protocols (SOPs) for immunohistochemistry - based detection of a panel of 6 biomarkers identified from the systems networks along with a robust scoring system for quantifying their

expression in FFPE sections of patient derived tumors and led to a successful validation of the predicted HGSC sub-types at the clinical level and revealed the influences of transcriptional heterogeneity on cellular plasticity and class-switching following chemotherapy. These findings are exciting since they not only validated predicted HGSC stratification at a clinical level, but support the future development of class-specific targeted therapy.

8. *Varankar SS, More MM, Abraham A, Kumar B, Narayanan NJ, Jolly MK, Bapat SA. Functional Balance between TCF21-Slug defines phenotypic plasticity and migratory modalities in high-grade serous ovarian cancer cell lines; Carcinogenesis. 2020 Jun 17;41(4):515-526.*

   Characterization of the HGSC molecular classes assigned Slug-driven EMT to one class, while another presented a strong epithelial identity that resisted EMT and expressed the epithelial transcription factor TCF21 (identified through systems networks analyses). In this study, the nominee identified novel regulatory cross-talks between Tcf21 and Slug that mediate phenotypic plasticity and differential modalities of cell migration in HGSC. Differential expression associated Tcf21 and Slug with epithelial and mesenchymal phenotypes respectively, while gene manipulation approaches resolved intermediate phenotypic states that implied a multistep EMT program. The balance of Tcf21–Slug expression and their differential subcellular localizations leading to a spectrum of cell phenotypes (epithelial - intermediate - hybrid – mesenchymal) was mirrored across a panel of HGSC cell lines. Further, microenvironment-induced plasticity and the preferential emergence of an epithelial phenotype following drug exposure through occupancy of Tcf21 at the Slug promoter was identified. This study provided a framework to understand HGSC subtypes, regulation of EMT and ovarian cancer cell plasticity as a function of two transcription factors.

9. *(i) Varankar SS, Bapat SA. Migratory Metrics of Wound Healing: A Quantification Approach for in vitro Scratch Assays. Front Oncol. 2018 Dec 18;8:633.*

   (ii) Varankar SS, Bapat SA. Uncoupling Traditional Functionalities of Metastasis: The Parting of Ways with Real-Time Assays. ***J Clin Med.*** *2019;8(7)pii:E941.*

   (iii) Varankar SS, Hari K, Bapat SA, Jolly MK*. Cell Geometry Distinguishes Migration-Associated Heterogeneity in Two-Dimensional Systems. Comput Syst Oncol. 2022; 2:e1041; * co-communicating authors.*

   Different modalities of cell migration associated with varying cell phenotypes was an extremely novel identification in a field that equates cancer cell invasion with EMT. Most endpoint assays rely on the efficacy of wound closure that thwarts quantification of migratory phenotypes observed during metastatic dissemination. The study performed by the nominee involved development of a protocol for live cell imaging of the classical wound healing assay and detailed analyses toward definition of three quantitative metrics viz. cell displacement, velocity and number of nearest neighbours, which provided global *vs.* single-cell resolution of migratory phenotypes as opposed to the classical endpoint assays. Importantly, this revealed that the epithelial subclass of HGSC incapable of undergoing EMT exhibited Cooperative Cell Migration (CCM) that revealed extensive proliferation leading to passive migration, which corroborates with the reported *in situ* passive shedding of cells from the surface of HGSC tumor into the peritoneal cavity. The intermediate and hybrid phenotypes exhibit an active CCM modality, reflected as sheet migration. Moving towards the mesenchymal end of the spectrum involves a combination of sheet migration and EMT which is the most efficient modality of migration, while mesenchymal cells associated with EMT alone achieved a slower rate of wound healing.

These findings provided a wealth of information of the metastatic capabilities of tumor cells through precise dissection of their molecular networks, and challenges the current dogma of performing end-point based invasion and migration assays in discerning nuances of the metastatic cascade. The above 3 reports emphasize that a complete comprehension of metastasis in view of evolving ideologies necessitates (i) the use of appropriate, context-specific assays and understanding their inherent limitations; (ii) cautious derivation of inferences to avoid erroneous/overestimated clinical extrapolations; (iii) corroboration between multiple assay outputs to gauge metastatic potential; and (iv) the development of protocols with improved in situ implications. Further adoption of mathematical modelling improved quantitative approaches in these assays and generated predictive algorithms that may expedite therapeutic strategies targeting metastasis via the development of disease relevant model systems. Such approaches could potentiate the restructuring of the cancer metastasis paradigm through an emphasis on the development of next-generation real-time assays.

10. *Kalra RS, Soman GS, Parab PB, Mali AM, Varankar SS, Naik RR, Kamble SC, Dhanjal JK, Bapat SA. A Monoclonal Antibody against Annexin A2 targets stem and progenitor cell fractions in tumors.* ***Translational Oncology*** *2022; 15(1):101257.*

Resolution of molecular subclasses provided an opportunity for the development of targeted treatments. A cytotoxic monoclonal antibody (termed as mAb150) was thus developed that recognizes a unique antigenic epitope in the Anxa2 protein with a high specificity. The association of Anxa2 with EMT is reported; additionally, it was suggested and validated as a biomarker for identification of the EMT subtype of HGSC tumors during clinical stratification. Tumor cells expressing AnxA2 were specifically targeted by mAb150, and treatment led to delayed invasion and migration through the aCCM and EMT modes. This assigned a cell phenotype- and tumor subclass- specific context to the efficacy of Anxa2 inhibition by mAb150. Further epigenetic potentiation of AnXA2 by 5-Aza-dC or HMATi in combination with mAb150 improved the efficacy of mAb150 and may deliver further prognostic benefits as is reported in immunotherapy. Therapeutic relevance of mAb150 was finally affirmed through findings in PDX models in which formation of ascites/intraperitoneal spheroids (marked by high AnxA2 levels) were significantly delayed along with extended survival in treated mice. This suggests that the monoclonal antibody can be used in a class-specific targeted manner for patients who present with tumors that stratify into the EMT class. An Indian patent # 374150 has been granted for mAb150 and the report published.

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