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> Blebbishields can fuse with immune cells, evading phagocytosis and generating hybrid cells with increased tumorigenesis, migration, and metastasis

The formation of a blebbishield and initiation of the blebbishield emergency programme by bladder cancer cells enable evasion of genomic and immune checkpoints, thus increasing chromosomal and genomic instability and avoidance of phagocytosis by apoptotic cancer stem cells.

The blebbishield emergency programme is a last defence by apoptotic cancer stem cells in which apoptotic cell membrane blebs fuse, forming a blebbishield that protects the cell and the nucleus from apoptosis. Blebbishields can fuse together, creating cancer stem cell spheres, causing cell transformation, and shifting the cells back towards survival.

The blebbishield emergency programme was believed to have two phases: phase one involves blebbishield construction, facilitating membrane fusion; phase two is the transformation phase, in which blebbishields fuse and cancer stem cell spheres are formed. New research indicates that a third phase, named the exit phase, exists. Time-lapse microscopy revealed that beyond 12–16 h transformed bladder cancer cell spheres (which had undergone the second phase) had a nucleoid centre and many peripheral nuclei. Furthermore, individual polarized cancer cells were released from the polarized front of the spheres. This phenomenon was also observed in prostate cancer cells.

Analysis of blebbishield transcriptome profiles revealed that transcripts are regulated in a stage-specific manner. Generation of a transformation-specific gene expression signature showed upregulation of transcripts related to stemness, cell fusion, apoptosis, adhesion, autophagy, malignancy, and xenobiotic metabolism. Further analysis showed that inhibition of RAP1 (which is required for reattachment of mitotic cells) blocked blebbishield transformation. Moreover, p53 expression was reduced in sphere-forming blebbishields, with corresponding increase in galectin 3 expression.

Co-culture of blebbishields with peripheral blood mononuclear cells (PBMCs) resulted in fusion with, rather than phagocytosis of, blebbishields. These fused blebbishields had upregulation of transcripts involved in genomic instability, cell survival, stemness, adhesion, migration, phagocytosis, metabolism, and proliferation. Furthermore, PBMC-fused blebbishields had increased chromosome numbers, surface CXC-chemokine receptor 4 expression, and chromosomal instability with morphologically identical chromosome sets (CIMICS), either complete set or incomplete set.

In vivo, these fused blebbishields caused more and larger tumours than RT4 bladder cancer cells and were associated with increased metastasis and worse prognosis. The blebbishield emergency programme caused increases in nuclear size, and giant nuclei contributed to the upregulation of genes in the transcription-specific signature. Blebbishields caused hepatosplenomegaly *in vivo*, and specimens expressed components of the blebbishield emergency programme, had reduced p53 and increased galectin 3 expression, and contained giant and multinucleated cells.

Together, these results suggest that the blebbishield emergency programme in apoptotic bladder cancer stem cells can bypass the p53-mediated genomic checkpoint, increasing chromosomal and genomic instability. This programme also results in cells with giant nuclei, which increases aggressiveness and tumorigenesis. Blebbishields can fuse with immune cells, evading phagocytosis and generating hybrid cells with increased tumorigenesis, migration, and metastasis. Furthermore, the blebbishield emergency programme has an exit phase in which the transformed spheres release daughter cells, some of which have genomic instability.

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