ESMO Symposium: Clinical Developments in the Diagnosis and Treatment of Solid Tumours

19 AUTOPHAGY INDUCTION AS A NEW THERAPY FOR HER2+ BREAST TUMORIGENESIS

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10.1136/esmoopen-2018-EACR25.19

Introduction HER2 is a receptor tyrosine kinase whose gene is amplified in ~20% human breast cancer patients. Allelic loss of the autophagy gene, beclin 1/BECN1, is associated with HER2 amplification in breast cancer; low beclin 1 mRNA expression is associated with increased risk of HER2-positive breast cancer; and overexpressed HER2 and Beclin 1 interact in cultured cells. However, the functional significance of HER2/Beclin 1 interaction and of altered autophagy in HER2-driven tumorigenesis and whether autophagy induction may be beneficial in preventing HER2-positive breast tumour growth is unknown.

Material and methods We explored the regulation of autophagy in breast cancer cells by HER2 in vitro and the effects as well as genetic and pharmacological approaches to increase autophagy on HER2-driven breast cancer growth in vivo.

Results and discussions Here we show that endogenous HER2 interacts with Beclin 1 in multiple HER2 +breast cancer cells and inhibits autophagy. Mice with a knock-in mutation in Beclin1 (Beclin1F121A)[1212] that leads to increased basal autophagy are protected from mammary tumorigenesis when crossed with mammary-specific HER2 transgenic mice, and HER2 fails to inhibit autophagy in primary cells derived from these mice.

Moreover, treatment of mice with HER2-positive human breast cancer xenografts with the Tat-Beclin 1 autophagy-inducing peptide inhibits tumour growth as effectively as a clinically used HER2 tyrosine kinase inhibitor (TKI). This inhibition of tumour growth is associated with a robust induction of autophagy, a disruption of HER2/Beclin 1 binding, and a transcriptional signature in the tumours that is distinct from that observed with HER2 TKI treatment.

Conclusion These findings indicate that the HER2-mediated inhibition of Beclin 1 and autophagy likely contributes to HER2-mediated tumorigenesis. They also suggest that strategies to block HER2/Beclin 1 binding and/or increase autophagy may represent a new therapeutic approach for HER2-positive breast cancers.

20 IDENTIFICATION OF COMBINATORIAL THERAPIES WITH INHIBITORS OF THE PI3K PATHWAY IN PTEN-NULL TUMOURS

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10.1136/esmoopen-2018-EACR25.20

Introduction PTEN is one of the most frequently mutated tumour suppressor genes in cancers including triple negative breast cancer (TNBC). PTEN loss results in the aberrant activation of PI3K signalling and in sensitivity to inhibitors of key components of the pathway, such as p110β and AKT. Nevertheless, the benefit of PI3K pathway inhibitors (PI3Kpi) in the clinical setting has so far been modest.

We aimed to identify genes whose inhibition potentiates the effects of PI3Kpi on PTEN-deficient TNBCs.

Material and methods We performed a genome-wide shRNA screening to identify genes that, when silenced, synergised with a p110β inhibitor, a AKT inhibitor or a pan-PI3K inhibitor in impairing the growth of MDA-MB-468 PTEN-null TNBC cell line.

We carried out a CRISPR-CAS9 screen on the top 144 hits to test whether their KO could suppress the phosphorylation of the downstream effector S6 in combination with PI3Kpi.

Results were validated using PTEN-deficient human cell lines with acquired resistance to PI3Kpi and a panel of TNBC cell lines with different PTEN status.

We also generated a mouse model in pure background in which the KO of Pten and p53 is restricted to the mammary glands to further characterise the synergisms in vivo.

Results and discussions We identified a number of hits whose blockage enhanced the growth inhibition and the phospho-S6 suppression induced by PI3Kpi. The onset of acquired resistance also correlated with an impaired suppression of phospho-S6 by PI3Kpi, pointing at S6 phosphorylation as a marker of treatment response in PTEN-null tumours.

The screenings identified EGFR, that is frequently overexpressed/amplified in TNBC, and GN2 and GNG3, which encode β and γ subunits of G protein.

The synergism between EGFR inhibition and PI3Kpi was validated in vitro by the use of approved drugs and the combination preferentially targeted PTEN-deficient TNBCs. Simultaneous inhibition of EGFR and p110β suppressed the growth of PTEN-deficient human tumour xenografts also in vivo with no sign of toxicity.

The KO of GB2 sensitised to the activity of pan-PI3Ki, increased EGFR phosphorylation and enhanced sensitivity to anti-EGFR drugs, pheno-copying and amplifying the effect of p110β blockage.

Conclusion We showed that EGFR and G proteins can orchestrate the sustained activation of the signalling downstream PI3K-AKT when PI3Kpi are applied to PTEN-null TNBCs. We envision that the identification of cues and GPCRs that act upstream G proteins and blunt the response to PI3K and EGFR inhibitors may unveil new therapeutic opportunities.

Symposium: Stem Cells

21 DEFINING THE CELL POPULATIONS RESPONSIBLE FOR SKIN CANCER INITIATION AND RELAPSE FOLLOWING THERAPY

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10.1136/esmoopen-2018-EACR25.21

Introduction The identification of specific cell type from which cancer arises and the cancer cell population that resists upon therapy leading to tumour relapse constitute the main
topics of our research. We use the basal cell carcinoma (BCC), the most frequent cancer in humans, as a cancer model for our studies.

**Material and methods** We used lineage tracing approach to elucidate the cell at the origin of BCC and study the cancer cell population responsible for relapse following therapy discontinuation.

**Results and discussions** To uncover the cancer cell of origin in BCC and the changes in the cellular dynamics that lead to tumour initiation, we assessed the impact of oncogenic hedgehog signalling activation in distinct cell populations and their capacity to induce BCC. We found that only stem cells, and not progenitors, were competent to initiate tumour formation upon oncogenic hedgehog signalling. Interestingly, this difference was due to the hierarchical organisation of tumour growth in oncogene-targeted stem cells, characterised by an increase of symmetric self-renewing divisions and a higher p53-dependent resistance to apoptosis, leading to rapid clonal expansion and progression into invasive tumours.1

I Sánchez-Danés A, Hannezo E, Larsimont JC, Liagre M, Youssef KK, Simons BD, Blanpain C. *Defining the clonal dynamics leading to mouse skin tumour initiation*. Nature. 2016 Jul 8;536(7616):298–303. doi: 10.1038/nature19069

To study the cancer cell population that mediates BCC relapse upon therapy, we treated two different genetic BCC mouse models with a Smoothened inhibitor (Smoi), the most commonly drug used to treat locally advanced and metastatic BCC. The mechanism by which SmoI leads BCC regression and emergence of resistant tumour cells are currently unknown. We found that SmoI mediates BCC regression by promoting epidermal differentiation and that during the course of SmoI administration, some BCC become resistant to therapy mimicking the situation found in humans. We identify Lgr5 as a marker expressed in the resistant tumour cell population upon SmoI administration. Finally, we demonstrate that combination of SmoI administration with Lgr5 lineage ablation leads to BCC eradication.

**Conclusion**

1. We demonstrated that stem cells cells are competent to initiate basal cell carcinoma, whereas, committed progenitors resistant.
2. We demonstrated that SmoI administration in combination with Lgr5 lineage ablation leads to BCC eradication.

**EVIDENCE FOR ANTIGEN-DRIVEN TCRB CHAIN CONVERGENCE IN THE TUMOURINFILTRATING T CELL REPERTOIRE OF 148 RESEARCH SUBJECTS WITH MELANOMA**

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**Introduction** T cell convergence refers to the phenomenon whereby antigen-driven selection enriches for T cell receptors having a shared antigen specificity but different amino acid or nucleotide sequence. T cell recruitment and expansion within the tumour microenvironment (TME) may be directed by responses to tumour neoantigen, suggesting that elevated T cell convergence could be a general feature of the tumour infiltrating T cell repertoire. Here we evaluate evidence for T cell convergence within tumour biopsy and peripheral blood leukocytes (PBL) from a set of 148 research subjects with melanoma.

**Material and methods** Total RNA from 85 tumour biopsy research samples (non-FFPE) was extracted for use in long-amplicon TCRB chain sequencing (mean amplicon length of 303. doi: 10.1136/nature19069

**Results and discussions** Transcriptional profiling showed that LSCmed is a distinct cell entity that exhibits a specific gene expression signature, among which cyto keratin 4 (CK4) was validated as a specific marker to track them on tissue sections. Using CK4 immunohistochemistry and cell sorting we discovered that LSCmed represent the major cell component (80%) of aggressive prostate tumours harboured by Pten-KO mice. LSCmed are castration-tolerant in the three genotypes, which correlates their intrinsically low androgen signalling. According to their progenitor properties, Pten-KO LSCmed exhibit cancer-initiating properties in transplantation assays, and generate more aggressive tumours than basal cells used in control. Finally, in Pten-KO prostate tumours, several clusters of LSCmed continue to proliferate after castration.

**Conclusion** LSCmed represent a newly-identified luminal prostate cell subpopulation. The combination of progenitor, castration-resistance and tumor-initiating properties makes them strong candidates for mediating prostate cancer recurrence under androgen deprivation therapy (CRPC).