Non-genetic rewiring of ERBB signaling in cancer drug tolerance

Iina Takala[1], Ph.D. Kari Kurppa[2] and Prof. Klaus Elenius[2]
[1]Department of Life Technologies, [2]Institute of Biomedicine, University of Turku

MOLECULAR BIOSCIENCES (Biochemistry)

INTRODUCTION

ERBB receptors

ERBB receptors are receptor tyrosine kinases (RTKs), transmembrane proteins that upon EGF-like ligand binding dimerize to homo- or heterodimers and become activate tyrosine kinases. Transphosphorylation of the C-terminal tails stimulates docking of downstream signaling molecules that cascade to pathways leading to cell growth and survival. Receptors stimulate different pathways, EGFR (epidermal growth factor receptor) being mitogenic, and neuregulin receptors ERBB2, ERBB3 and ERBB4 promoting survival.

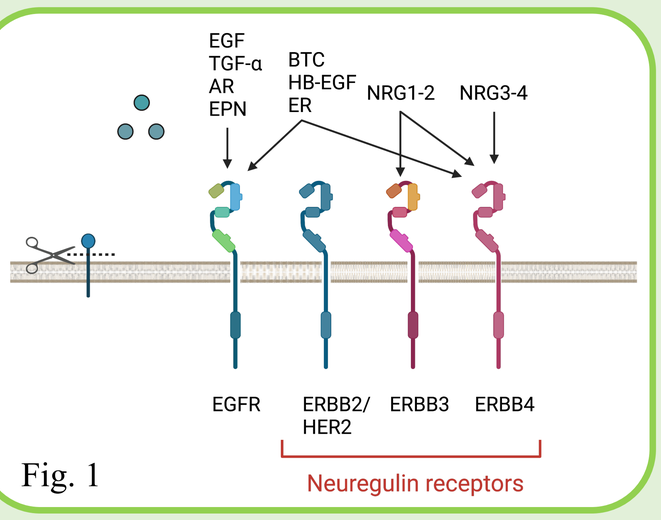

Fig. 1

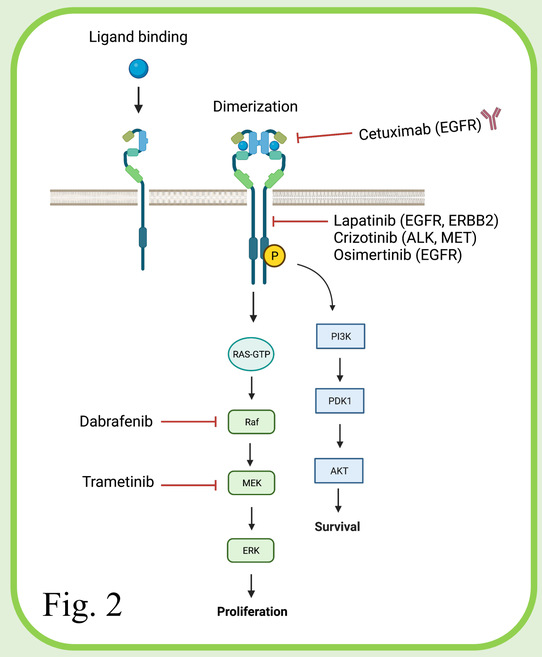

Fig. 2

Drug tolerance

RTKs are a common target for cancer therapy, and a range of tyrosine kinase inhibitors (TKIs) and antibodies that target them are in clinical use (Fig. 2). Despite of successful results in the targeted therapy, many patients experience relapse of the disease after certain time, even after full clinical clearance of cancer. This is due to minimal residual disease (MRD), a condition where drug tolerant persister cells (DTPs) remain in the patient through the treatment in dormant state. After extended time of treatment they begin cycling and are prone to gain mutations that eventually lead to the emergence of cancer drug resistance (Fig. 3), the main reason that makes advanced cancer an uncurable disease.

Aim & hypothesis

In this project, the aim was to define and understand the early events of cancer drug tolerance through expression of ERBB receptors and their ligands. Hypothesis of the study was that ERBB RTKs and neuregulins would be induced upon drug treatment. Drug treatment would increase expression which contributes to rewiring of cancer cell signaling in order to become drug tolerant.

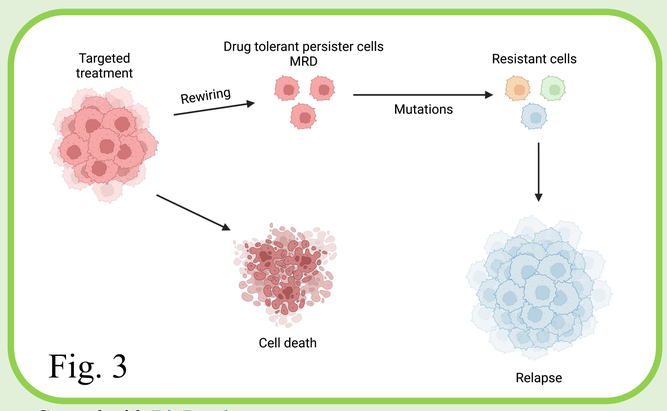

Fig. 3

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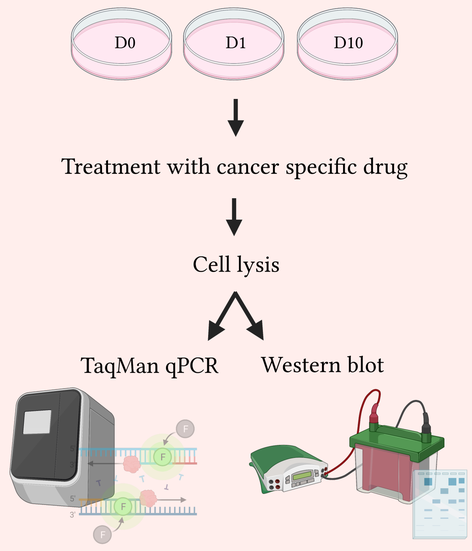
METHODS

Treatment of five different cell lines (A375, H3122, BT474, HCC827 and HT-29) for 0, 1 and 10 days (DTPs)
Two rounds to qPCR, one round to western blot

qPCR: Cell lysis, RNA extraction, cDNA synthesis → TaqMan qPCR from cDNA template with gene specific primers and probes to detect CT values of each gene and β-actin reference gene

Western blot: Cell lysis, 10% SDS-PAGE, blot, incubation in receptor specific primary antibody, detection of signal with fluorescent IR DYE® 800 CW secondary antibody (LI-COR



	A375			H3122			BT474			HCC827			HT-29		
	D0	D1	D10	D0	D1	D10	D0	D1	D10	D0	D1	D10	D0	D1	D10
EGFR	0.0	-1.9	-1.3	0.0	-0.2	0.0	0.0	1.5	0.2	0.0	0.8	0.3	0.0	-0.6	0.4
ERBB2	0.0	2.1	2.3	0.0	1.6	2.7	0.0	1.0	-0.2	0.0	1.6	2.7	0.0	2.3	2.5
ERBB3	0.0	1.9	1.8	0.0	1.4	2.4	0.0	2.0	1.5	0.0	2.2	2.8	0.0	2.7	2.5
ERBB4	0.0	4.2	4.7	0.0	1.5	3.6	0.0	2.6	1.1	0.0	1.2	3.0	0.0	4.2	3.0
EGF	0.0	1.8	1.4	0.0	-0.4	0.2	0.0	0.9	-1.1	0.0	1.1	1.0	0.0	0.6	0.1
TGF-a	0.0	-3.4	-4.3	0.0	-0.1	1.2	0.0	1.0	1.1	0.0	0.2	1.0	0.0	0.3	0.9
AR	0.0	-5.0	-6.5	0.0	-2.7	-3.6	0.0	0.4	1.8	0.0	-0.9	-3.0	0.0	-0.6	-0.9
BTC	0.0	3.6	6.7	0.0	0.0	0.8	0.0	1.6	1.1	0.0	0.8	-0.1	0.0	1.8	2.0
HB-EGF	0.0	-1.3	-1.5	0.0	-1.5	1.2	0.0	2.0	0.2	0.0	1.8	3.7	0.0	-1.0	-0.1
ER				0.0	-4.3	-7.8	0.0	0.7	1.1	0.0	-0.7	-2.9	0.0	-1.2	-1.6
NRG-1	0.0	-0.7	-0.8	0.0	-1.7	1.6				0.0	0.8	-0.2			
NRG-2	0.0	-0.3	-0.5				0.0	4.8	3.8						
NRG-3	0.0	1.4	1.0				0.0	0.5	1.4						
NRG-4	0.0	4.1	-0.8	0.0	-0.6	-2.0	0.0	2.7	2.1	0.0	1.3	0.1	0.0	3.8	4.0

Fig. 4 Log2 of fold changes in gene expression

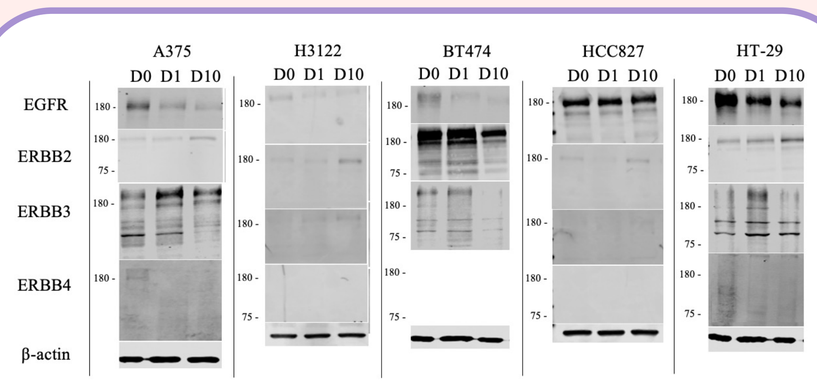


Fig. 5 ERBB protein expression

RESULTS

In the cell lines studied, a pattern of neuregulin receptor induction upon treatment was observed in the qPCR experiments. These receptors include the ERBB2, ERBB3 and ERBB4 receptors, and in nearly all the samples the fold change of these genes was increased, meaning an upregulation of expression (Fig. 4). Similarly, a pattern-like behaviour was observed in the EGFR receptor throughout the different cell lines, where its expression was downregulated or remaining stable. In the ligand expression, we observed an increase in at least one neuregulin isoform expression in each cell line, and a consistent downregulation of the EGFR ligand amphiregulin (AR).

Western blot experiments (Fig. 5) showed a similar increase pattern in ERBB2 and a decrease in EGFR. Also ERBB3 seemed to show an induction especially on the day 1 samples, but exhibited a lot of background. ERBB4 expression was in such low levels that it was not possible to observe these patterns.

	D0	D1	D10
EGFR	0.0	-0.1	-0.1
ERBB2	0.0	1.7	2.0
ERBB3	0.0	2.0	2.2
ERBB4	0.0	2.7	3.1

CONCLUSIONS

- Induction of neuregulin receptors ERBB2, ERBB3 and ERBB4 promotes cell survival pathway which benefits the cells to persist under treatment conditions
- Neuregulin expression contributes to the autocrine stimulation of the pathway