

Novel molecular features associated with sensitivity to the anticancer effects of statins in breast tumour cells

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ABSTRACT

It is quite remarkable that statins, drugs prescribed for decades to treat hypercholesterolemia, are now also proving to have promising anticancer activity. In several tumour types, these drugs have been shown to induce apoptosis by inhibiting the mevalonate (MVA) pathway, without harming normal cells. In particular, several epidemiological, preclinical and clinical studies have suggested that certain breast tumours may be susceptible to statin treatment. Furthermore, we have shown that high expression of genes involved in the MVA pathway in primary breast tumours is associated with poor prognosis (Clendening *et al.* PNAS, 2010). As breast cancer is a quite heterogeneous disease, an understanding of molecular features predictive of relative sensitivity or resistance to the anticancer effects of statins is imperative.

We hypothesize that statin-sensitive breast tumour cells may have aberrant regulation of the MVA pathway, along with several other novel molecular hallmarks. A panel of 19 heterogeneous breast cancer cell lines was assessed for sensitivity to the antiproliferative effects of fluvastatin and lovastatin by MTT assay. Clear delineations between relatively statin-sensitive and insensitive cell lines emerged, with sensitive cell lines undergoing apoptosis in response to treatment with either statin. In addition, statin-treated sensitive cell lines exhibited a reduction in oxygen consumption, as assessed on the XF96 analyzer (Seahorse Bioscience). Interestingly, evaluation of HMGR protein and mRNA levels indicated that classic sterol feedback regulation occurred in both statin-sensitive and insensitive cell lines. This suggests that dysregulation of sterol feedback in the MVA pathway is not predictive of statin sensitivity in breast cancer.

We also found that statin sensitivity did not appear to segregate with several common histopathological features. Intriguingly, however, an association with estrogen receptor (ER) status did emerge, with statin-sensitive cells being ER negative, with a non-luminal molecular subtype. This may indicate that ER signaling contributes to statin resistance.

To complement these observations, we have identified genes whose basal mRNA expression correlates with statin sensitivity, identifying genes involved in protein translation and cell motility. Mining this data has also allowed us to generate a preliminary molecular signature predictive of statin sensitivity. Taken together, this research will provide us and others with molecular features associated with sensitivity to the anticancer effects of statins in breast cancer. This will prove valuable since statins are FDA-approved drugs and can hence be fast-tracked to clinical trials in a subset of breast cancer patients enriched for those predicted to be responsive to statin therapy.

INTRODUCTION

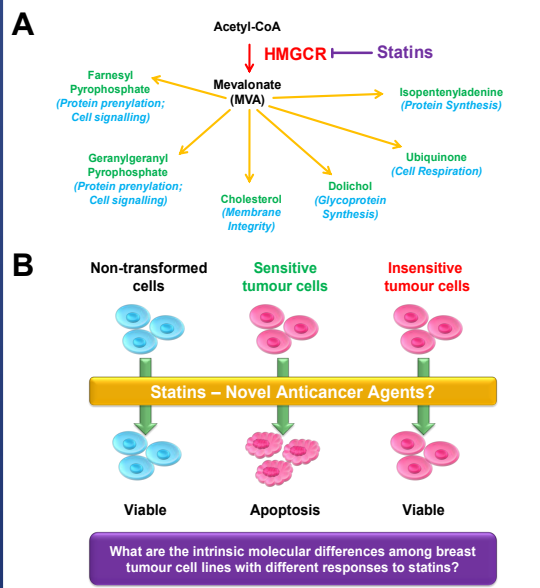


Figure 1. Statins have promising anticancer properties, and we must understand differential statin sensitivity among tumours to exploit statins as novel anticancer therapeutics. **A.** Statins induce tumour-selective apoptosis by inhibiting HMGR, the rate-limiting enzyme of the mevalonate (MVA) pathway. **B.** While statins lack general cytotoxicity, not all tumour types respond to their anticancer effects. Substantial heterogeneity in sensitivity also exists among tumours or cell lines of a given tumour type.

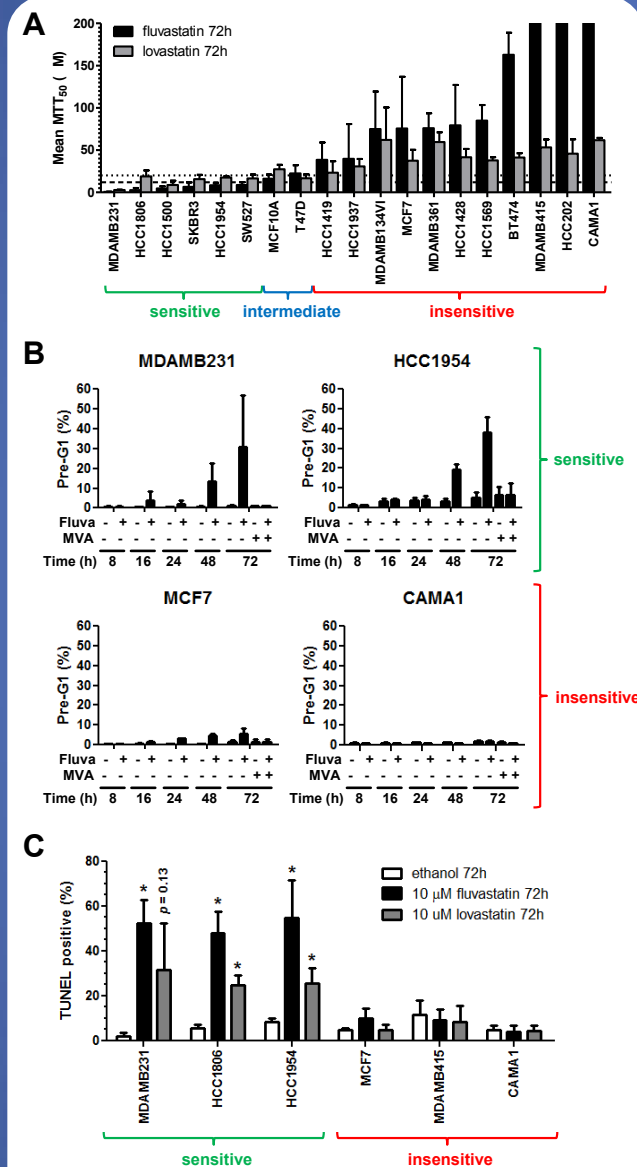


Figure 2. Breast cell lines exhibit a heterogeneous response to statins.

A. Statin sensitivity was measured by MTT assay. Mean MTT_{50} values, referring to the dose of statin at which 50% of mitochondrial dehydrogenase activity (a proxy for cell viability) is inhibited in 72 h, are presented with error bars indicating SD, for 3 to 7 independent experiments. Cell lines were considered sensitive if the mean MTT_{50} of at least one statin was $< 12 \mu\text{M}$ (predicted to be clinically achievable in plasma, dashed line), and insensitive if the MTT_{50} values of both statins were $> 20 \mu\text{M}$ (commonly used *in vitro*, dotted line). Cell lines where $12 \mu\text{M} < MTT_{50} < 20 \mu\text{M}$ were considered to have intermediate statin sensitivity. **B.** Statin-sensitive cell lines undergo cell death upon treatment with $10 \mu\text{M}$ fluvastatin. This was measured by pre-G1 DNA content in the fixed propidium iodide assay, detected by flow cytometry. This death is reversible by the addition of $200 \mu\text{M}$ exogenous mevalonate. Bars represent means of 2 independent experiments, with error bars indicating SD. Fluva, fluvastatin; MVA, mevalonate. **C.** Statin-sensitive cells undergo apoptosis upon treatment with statins. Cells were treated for 72 h then fixed and assessed for apoptosis by TUNEL staining, detected by flow cytometry. Bars represent means of 3 independent experiments, with error bars indicating SD. Asterisks indicate $p < 0.05$ in a two-sample, two-tailed, heteroscedastic t-test comparing statin-treated to ethanol vehicle-treated cells.

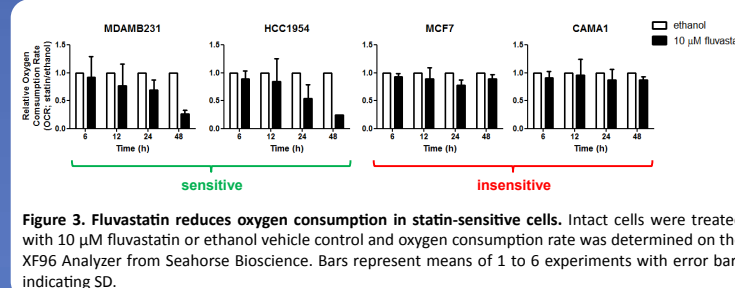


Figure 3. Fluvastatin reduces oxygen consumption in statin-sensitive cells. Intact cells were treated with $10 \mu\text{M}$ fluvastatin or ethanol vehicle control and oxygen consumption rate was determined on the XF96 Analyzer from Seahorse Bioscience. Bars represent means of 1 to 6 experiments with error bars indicating SD.

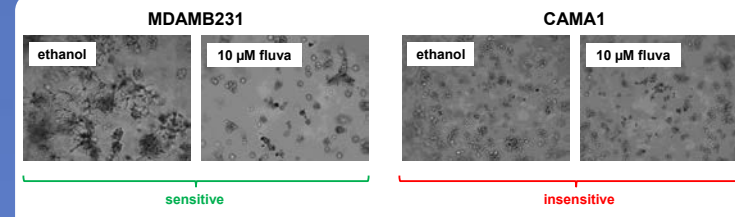


Figure 4. Relative statin sensitivity is preserved in 3D culture. Cells were grown for 4 days on matrigel, and were then treated for 72 h with $10 \mu\text{M}$ fluvastatin (fluva) or ethanol vehicle control. Bright field images were taken using a Zeiss AxioObserver. Images shown are representative of 2 to 3 experiments.

Table 1. Statin sensitivity may be associated with estrogen receptor (ER) status.

Cell Line	Statin Sensitivity Classification	Mean MTT_{50} Fluvastatin (μM)	Mean MTT_{50} Lovastatin (μM)	As assessed by Neve <i>et al.</i> (2006)				Molecular Subtype
				ER Status*	PR Status	HER2 Status	p53 Expression Status	
MDAMB231	sens	0.7	2.8	-	-	-	++	BaB
HCC1806	sens	2.7	18.8	ND	ND	ND	ND	ND
HCC1500	sens	4.7	8.8	-	-	-	-	BaB
SKBR3	sens	6.4	15.6	-	-	+	+	Lu
HCC1954	sens	8.5	17.5	-	-	+	+/-	BaA
SW527	sens	8.7	16.6	ND	ND	ND	ND	ND
MCF10A	intermed	16.2	27.3	-	-	-	+/-	BaB
T47D	intermed	22.4	16.7	+	+	-	++	Lu
HCC1419	insens	38.6	23.2	ND	ND	ND	ND	ND
HCC1937	insens	39.8	30.8	-	-	-	-	BaA
MDAMB134VI	insens	74.9	62.1	+	-	-	+/-	Lu
MCF7	insens	75.7	37.6	+	+	-	+/-	Lu
MDAMB361	insens	75.9	59.6	+	-	-	-	Lu
HCC1428	insens	79.4	41.6	+	+	-	+	Lu
HCC1569	insens	84.9	37.8	-	-	+	-	BaA
BT474	insens	162.8	41.2	+	+	+	+	Lu
MDAMB415	insens	ND	53.3	+	-	-	+	Lu
HCC202	insens	ND	45.8	-	-	+	-	Lu
CAMA1	insens	ND	61.8	+	-	-	+	Lu

* ER status is significantly associated with lovastatin sensitivity ($p = 0.008$), and a trend is observed toward association with fluvastatin sensitivity ($p = 0.07$), by point-biserial correlation.
ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; sens, sensitive; intermed, intermediate; insens, insensitive; BaA, basal A; BaB, basal B; Lu, luminal; ND, not determined.

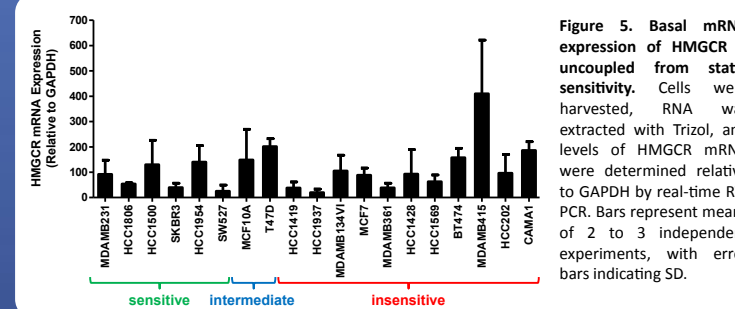


Figure 5. Basal mRNA expression of HMGR is uncoupled from statin sensitivity. Cells were harvested, RNA was extracted with Trizol, and levels of HMGR mRNA were determined relative to GAPDH by real-time RT-PCR. Bars represent means of 2 to 3 independent experiments, with error bars indicating SD.

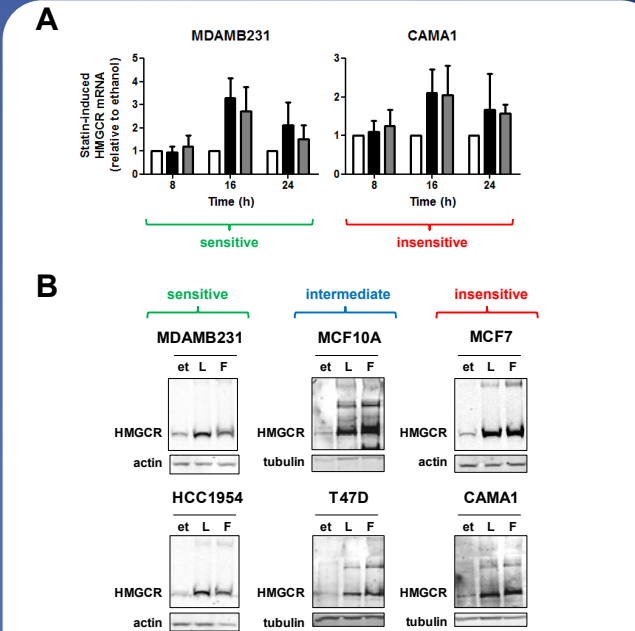


Figure 6. Statin-induced sterol feedback control of HMGR appears to be intact in both statin-sensitive and insensitive breast tumour cell lines.

A. HMGR mRNA expression is induced by statin treatment in both sensitive and insensitive cell lines. Following treatment, HMGR mRNA expression was assessed relative to GAPDH by real-time RT-PCR. Bars represent means of 3 to 4 independent experiments, with error bars indicating SD. **B.** HMGR protein expression is also induced by statin treatment regardless of sensitivity. Following treatment with ethanol (et), $10 \mu\text{M}$ fluvastatin (F) or $10 \mu\text{M}$ lovastatin (L) for 48 h, HMGR protein levels were assessed by immunoblot with the monoclonal antibody A9. Images are representative of 2 to 3 independent experiments.

CONCLUSIONS & FUTURE WORK

To our knowledge, this is the first extensive assessment of statin sensitivity in a large panel of heterogeneous breast tumour cell lines. To date, we have learned:

- Breast tumour cell lines display heterogeneous responses to the antiproliferative effects of two statins.
- Sensitivity to statins in breast tumour cells may be correlated with a lack of estrogen receptor expression.
- Relative statin sensitivity seems to be preserved in both 2D and 3D conditions.
- Statin sensitivity does not appear to be associated with a loss of HMGR feedback regulation at the mRNA or protein level.

To build upon this work, we have immediate plans to:

- Develop both basal and statin-induced global gene expression signatures predictive of statin sensitivity and indicative of response, respectively.
- Further examine the role of dysregulation of the MVA pathway and its components in statin sensitivity.

Ultimately, we believe that this work brings us one step closer to understanding which breast tumours will respond to statin therapy in a clinical setting.

