# Suitability of cell lines as a study model for 5-hydroxymethylcytosine

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## BACKGROUND

- 5-hydroxymethylcytosine (5-hmC)
  - → first transformation in demethylation process
  - → catalysed by ten-eleven-translocation (TET) protein
  - → distributed in genes bodies, enhancers, and in sequences with lower CpG contents[1]

**Table 1. Role of 5hmC in cancer**

| Type of Cancer | Mechanism/Hypothesis for 5-hmc loss |
|---|---|
| Brain | IDH1/2 mutations, Nuclear exclusion of TET1[2] |
| Breast | Increased Mir-22 expression[3], Decreased TET1/2/3 expression[4], Defective RARβ/TET2 signalling[5] |
| Colon | Decreased TET1 expression[6] |
| Gastric | Decreased IDH2, TET1,2,3 expression[7] |
| Blood | TET2 mutations[8] |
| Head and neck | Decreased TET2 expression[9] |

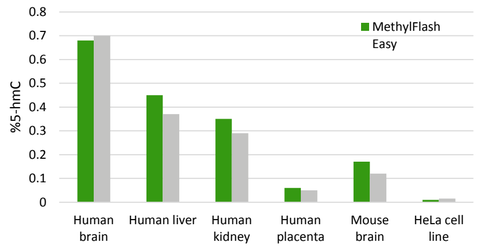
- Decrease of 5-hmC has been proposed as a marker for poor prognosis in the ER/PR-negative breast cancer subtype[10].
- Hypoxia is a hallmark of developing solid malignancies, including breast cancer[11], and an essential in the oxygen-dependent conversion of 5hmC[1].
- Hypoxia has been shown to induce loss of 5-hmC in the DNA of a panel of cancer cells[12].
- A previous study highlighted a loss of 5-hmC in primary fibroblast grown in plastic[13].

## METHODS

**Cell lines:**

MCF10-A (non carcinogenic mammary epithelial cells)
MCF-7 (Luminal A phenotype, ER+, PR+, HER2-)
BT474 (Luminal B phenotype, ER-, PR+, HER2+)
SKBR3 (HER2 enriched phenotype, ER-, PR-, HER2+)


**0.1% $O_2$ 24 hours**
**Whitley H35 Hypoxystation**




**Comparison between MethylFlash™ Global DNA Hydroxymethylation Assay and MS-LC (from company website)**

**5hmC abundance:** Colourimetric assay (MethylFlash™ Hydroxymethylated DNA Quantification Kit, EPIGENTEK)

***TET1, 2, 3 mRNA* expression:** qPCR: SYBR Green (reference genes: *HPRT1* and *POLR2A)*

## CONCLUSION

- The use of breast cell lines for the study of hydroxymethylation should be critically assessed.
- Breast cell lines are still a valuable tool to study the upstream mechanisms involved in this epigenetic modification.

## REFERENCES

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## AIM

- To determine how hypoxia regulates 5-hmC in the DNA and TET mRNA expression in a panel of breast cancer cell lines
- To determine if breast cell lines can be used as a model for 5-hmC studies

## RESULTS

- Hypoxia does not induce changes in 5-hmC in the DNA of normal breast cells.
- **Levels of 5-hmC detectable *in vitro* are negligible and, in cancer cells, not consistent amongst repeats.**

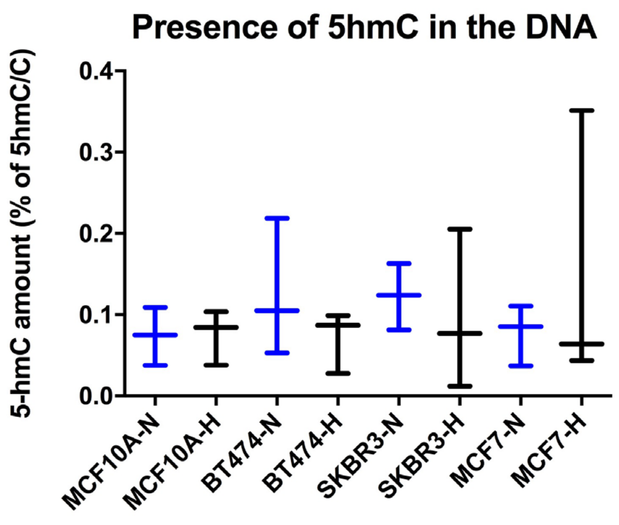


**Figure 1.** Our chosen assay estimates ~0.1% of the total methylated cytosines are hydroxylated in the DNA of the breast cell lines (comparable with other assays). Interestingly, cancer cells show significant variation of 5-hmC amongst three independent biological repeats, partly confirming previous findings on mouse fibroblasts.

- *TET1* mRNA downregulation in ER+/PR+/HER2- cell line MCF7 and in the ER-/PR+/HER2+ cell line BT474 is hypoxia-independent.
- **Hypoxia induces downregulation of TET2 mRNA in the normal cell line MCF10A, but not in any of the tumorigenic cell lines.**
- The expression of TET3 mRNA is not significantly different amongst the four cell lines, independently of oxygen levels.

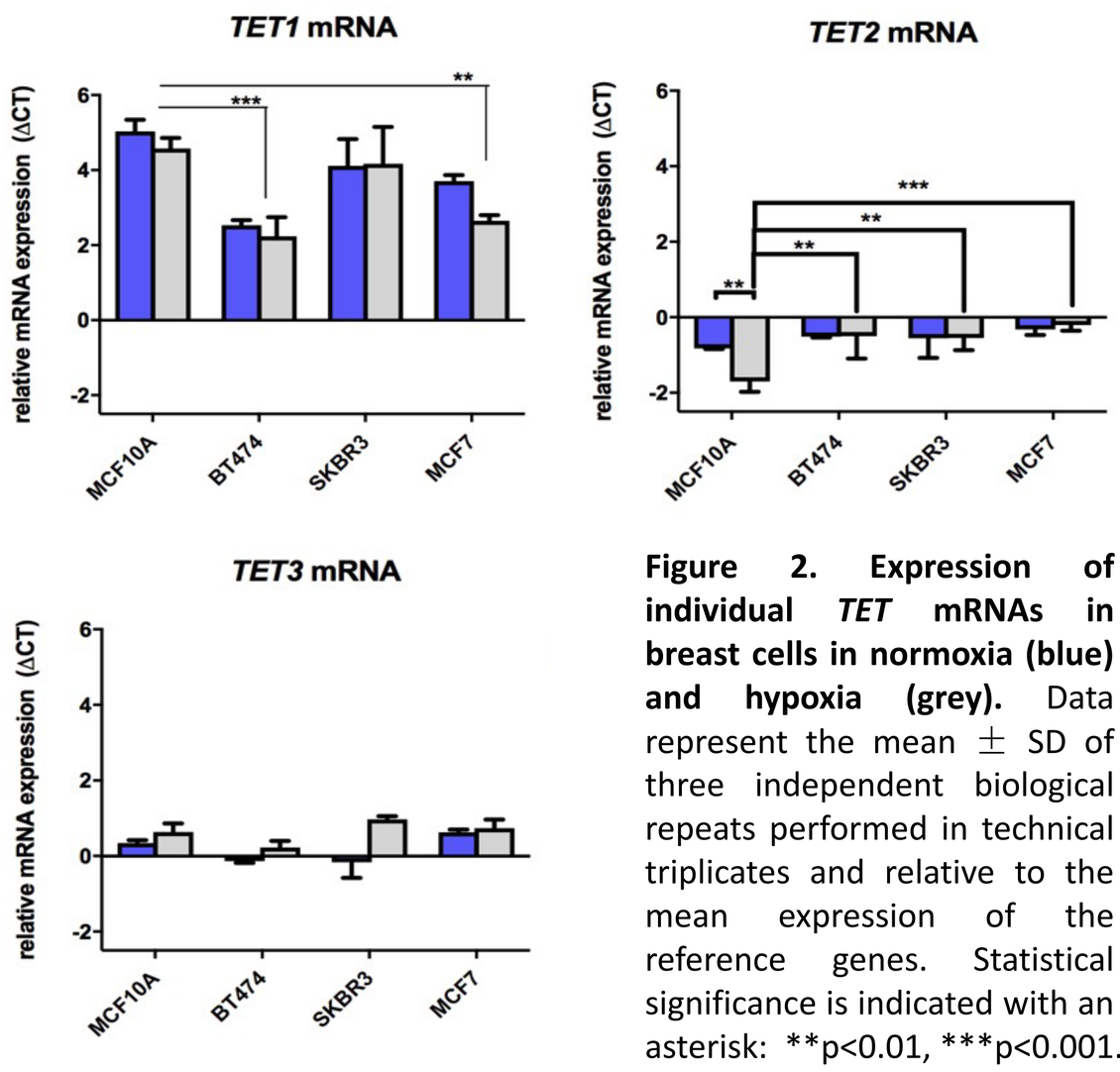


**Figure 2. Expression of individual *TET* mRNAs in breast cells in normoxia (blue) and hypoxia (grey).** Data represent the mean ± SD of three independent biological repeats performed in technical triplicates and relative to the mean expression of the reference genes. Statistical significance is indicated with an asterisk: **p<0.01, ***p<0.001.