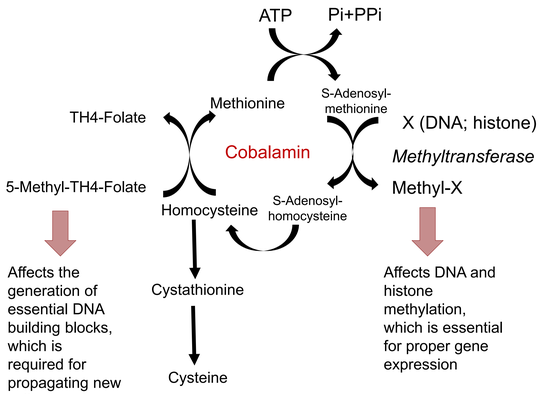
Reassessing cobalamin requirements in cell culture

David J. Elzi[1], Reggie Jacob[1], and Vivienne I. Rebel[1]

Introduction

- Cobalamin (Vitamin B12) is an essential co-factor for the synthesis of components required for cell growth and maintenance
- Cobalamin is important for one-carbon metabolism which is involved with genome methylation and nucleotide synthesis
- Cancer cells have increased metabolic needs
- We hypothesized that cancer cell proliferation would be negatively affected if grown in cobalamin-deficient medium

Cobalamin metabolism



Development of defined growth medium

- DMEM-Serum free medium (SFM) is based on DMEM:F12 components
- DMEM-high glucose is used as the base medium, which contains no cobalamin

Table1. Components added to DMEM to make DMEM-SFM

Trace elements	Amino acids	Vitamins and other	Lipids and related	Growth factors
Copper sulfate-5H_2O	100x NEAA	D-biotin	Choline chloride	3,3',5-Triiodo-L-thyronine
Ferrous sulfate-7H_20	L-Arginine monohydrochloride	Cobalamin	Linoleic acid	Epidermal growth factor
Zinc sulfate-7H20	L-Asparagine monohydrate	Hypoxanthine disodium salt	(±)-α-Lipoic acid	Hydrocortisone
Selenium	L-Cysteine hydrochloride monohydrate	Thymidine	Putrescine dihydrochloride	Insulin
	L-Proline	myo-Inositol	O-Phosphorylethanolamine	Basic fibroblast growth factor (only for U251and PC3 cells)
			Lipid mixture chemically defined	Transferrin

- MEM-SFM was based upon MEM basal medium
- MEM base medium has no added cobalamin

Table 2. Components added to MEM to make MEM-SFM

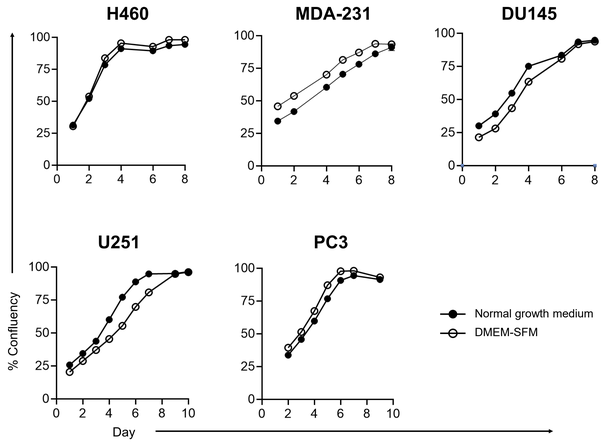
Trace elements	Vitamins and other	Lipids and related	Growth factors
Selenium	Cobalamin	Lipid mixture chemically defined	Insulin
	Sodium Pyruvate		Transferrin

ELISA measurements of cobalamin levels in reagents

- A commercial ELISA kit was used to determine cobalamin levels in the medium and subculture reagents
- The ELISA had a detection limit of 73 pg/ml cobalamin

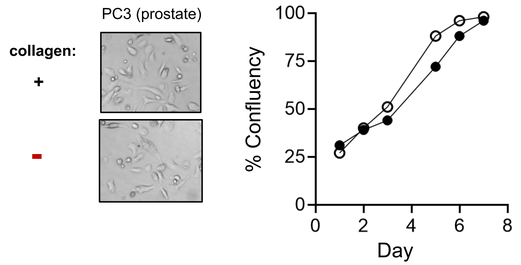
Item	pg/ml cobalamin
Fetal Bovine Serum	224
Rat Tail Collagen	116
Poly-lysine	below detection limit
100x ITS	below detection limit
100x CD-lipids	below detection limit
Trypsin-EDTA	below detection limit
TNS	below detection limit
HBSS	below detection limit
DMEM-SFM	below detection limit
MEM-SFM	below detection limit

Multiple cell lines grow in defined medium



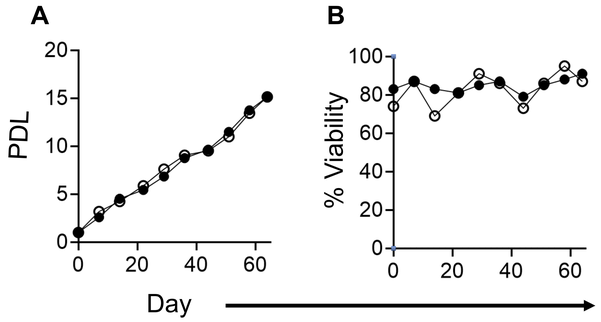
Note: All cells were grown on collagen-treated dishes, which has a trace amount (~ 2.23 x 10^{-12} M) of cobalamin.

PC3 cells grow under minimal cobalamin growth conditions



PC3 cells were able to grow without collagen substrate. PC3 cells were grown in DMEM-SFM with 1.65x10^{-11}M cobalamin (black circles) or without cobalamin (white circles).

NAMALWA growth not affected by cobalamin restriction



NAMALWA cells were grown in MEM-SFM with 1.65x10^{-11}M cobalamin (black circles) or without cobalamin (white circles). A – Cell growth was determined by population doubling level (PDL). B – Viability was determined by trypan blue stain.

Conclusions

- Cancer cell lines grew in growth medium with at most 5.38 x 10^{-11} M cobalamin, which is the detection limit of the cobalamin ELISA assay used in our studies
- Cobalamin-restriction studies should be mindful of trace amounts of cobalamin in cell culture components, which are difficult to measure with most cobalamin detection assays
- Cobalamin detection assays with a lower limit of detection would allow for a more accurate measurement of cobalamin levels in tissue culture reagents, which may lead to a better understanding of cobalamin requirements for cell growth

CELL BIO
An ASCB | EMBO Meeting
Dec. 3-7, 2022 • Washington, DC

Poster presentation: P1973
Board number: B291

[1]bioAffinity Technologies, San Antonio, TX, USA