DIETARY PROTEIN RESTRICTION OF PREGNANT MOUSE AND SUSCEPTIBILITY TO THE DEVELOPMENT OF CHEMICALLY-INDUCED ESOPHAGEAL AND LIVER CANCER

Sampaio, ARD[1]; Blanco T, PN[2]; Lima, SCS[2]; Pinto, LFR[1,2]

[1]Departamento de Bioquímica – IBRAG – Universidade do Estado do Rio de Janeiro, Brazil; [2]Programa de Carcinogênese Molecular – CPQ – INCA, Brazil.

INTRODUCTION

The term "developmental programming" has been used to describe the process whereby insults received during critical periods of development can generate permanent changes in body structure and function that affect the homeostasis of specific organs in the adult life[1,2,3].

This phenomenon has been integrated into to the developmental origins of health and disease (DOHaD) hypothesis, which suggests an association between mammalian prenatal environmental exposure and subsequent risk of developing non communicable chronic diseases[4]. However, the relation between this hypothesis and cancer is still sparse in the literature.

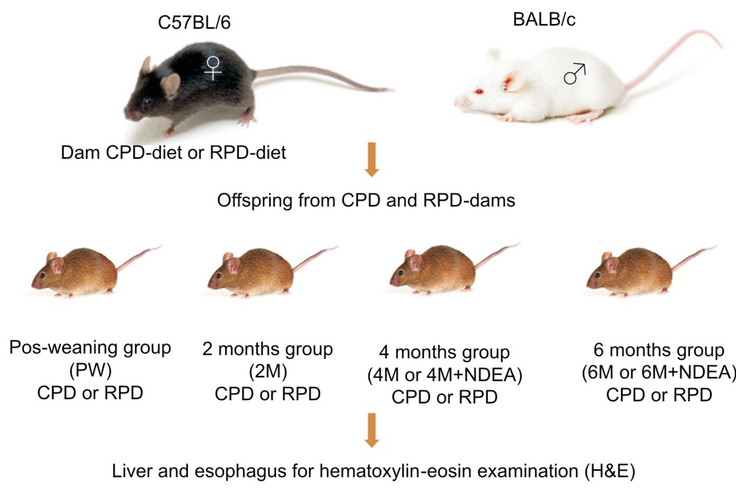
In several rodent models, in utero exposure to low protein diet leads to metabolic diseases in the adult offspring[3,5,6]. Now, emerging data also supports it in relation to mammary carcinogenesis process[7,8,9]. Nevertheless, there's no study on "programming", using low protein gestational diet in esophageal and liver carcinogenesis.

OBJECTIVE

To investigate the effect of low protein maternal diet on the susceptibility of developing chemically-induced esophageal and liver cancer in adult offspring.

METHODOLOGY

Female mice were mated with male mice in a 1:1 mating scheme and the beginning of gestation was determined via assessment of vaginal plug expellation. The pregnant dams were fed *ad libitum* a control protein diet (CPD-17% protein) or a restricted protein diet (RPD-8% protein), throughout pregnancy. After weaning, all pups received standard diet and were divided in groups with or without N-nitrosodiethylamine (NDEA 40 ppm) *ad libitum* in drinking water.



RESULTS

Summary of histological analysis of esophagus from male mice treated or not with NDEA 40 ppm.

Group		4M+NDEA		4M		6M+NDEA		6M	
		CPD	RPD	CPD	RPD	CPD	RPD	CPD	RPD
N		5	5	5	5	5	5	5	5
Mild inflammation - foci	0	-	-	5 (100%)	5(100%)	-	-	5(100%)	5 (100%)
	1 - 3	2 (40%)	1(20%)	-	-	-	-	-	-
	4 - 6	3 (60%)	4 (80%)	-	-	1 (20%)	1 (20%)	-	-
	7 - 9	-	-	-	-	-	2 (40%)	-	-
	10 - 12	-	-	-	-	3 (60%)	2 (40%)	-	-
	13 - 15	-	-	-	-	1 (20%)	-	-	-
AHD	1	-	-	-	-	-	1 (20%)	-	-
	2	-	-	-	-	2 (40%)	1 (20%)	-	-

AHD – atypical hyperplasia with dyskeratosis; CPD – dam control protein diet (17%); RPD – dam restricted protein diet (8%).

Summary of histological analysis of esophagus from female mice treated or not with NDEA 40 ppm.

Group		4M+NDEA		4M		6M+NDEA		6M	
		CPD	RPD	CPD	RPD	CPD	RPD	CPD	RPD
N		5	5	5	5	5	5	5	5
Mild inflamation - focus	0	1 (20%)	-	5 (100%)	5 (100%)	-	-	5 (100%)	5 (100%)
	1 - 3	1 (20%)	3 (60%)	-	-	-	-	-	-
	4 - 6	3 (60%)	2 (40%)	-	-	2 (40%)	2 (40%)	-	--
	7 - 9	-	-	-	-	3 (60%)	3 (60%)	-	-
	10 - 12	-	-	-	-	-	-	-	-
	13 - 15	-	-	-	-	-	-	-	-
AHD	1	-	-	-	-	-	1 (20%)	-	-
	2	-	-	-	-	-	-	-	-

AHD – atypical hyperplasia with dyskeratosis; CPD - dam control protein diet (17%); RPD – dam restricted protein diet (8%).

- Esophagus from PW (CPD; RPD) and 2M (CPD; RPD) mice presented normal morphology.

Summary of histological analysis of liver from male mice treated or not with NDEA 40 ppm.

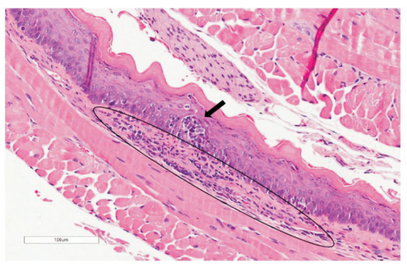
Group	Morphological phenotype	Animals (n)
4M+NDEA (CPD) (n = 5)	A	4 (80%)
	B, C, D	1 (20%)
4M+NDEA (RPD) (n = 5)	A	4 (80%)
	B, C, D	1 (20%)
4M (CPD) (n = 5)	A	5 (100%)
4M (RPD) (n = 5)	A	5 (100%)
6M+NDEA (CPD) (n = 5)	B, C, D	4 (80%)
	B, C, D, E	1 (20%)
6M+NDEA (RPD) (n = 5)	B, C, D	4 (80%)
	B, C, D, E	1 (20%)
6M (CPD) (n = 5)	A	5 (100%)
6M (RPD) (n = 5)	A	5 (100%)

Summary of histological analysis of liver from female mice treated or not with NDEA 40 ppm.

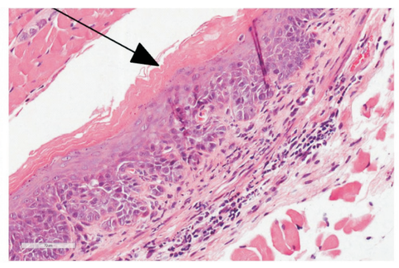
Group	Morphological phenotype	Animals (n)
4M+NDEA (CPD) (n = 5)	B, D	1 (20%)
	C, D	1 (20%)
	B, C, D	3 (60%)
4M+NDEA (RPD) (n = 5)	B, C, D	5 (100%)
4M (CPD) (n = 5)	A	5 (100%)
4M (RPD) (n = 5)	A	5 (100%)
6M+NDEA (CPD) (n = 5)	B, C, D, E	5 (100%)
6M+NDEA (RPD) (n = 5)	B, C, D, E	5 (100%)
6M (CPD) (n = 5)	A	5 (100%)
6M (RPD) (n = 5)	A	5 (100%)

A – Normal liver tissue; **B** – Proliferation of elongated cells; **C** – Proliferation of tubule-glandular structures without atypia; **D** – Proliferation of tubule-glandular structures with low grade atypia; **E** - Proliferation of tubule-glandular structures with high grade atypia.

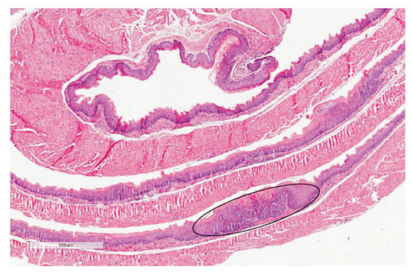
- Livers from PW (CPD; RPD) and 2M (CPD; RPD) mice presented normal morphology.



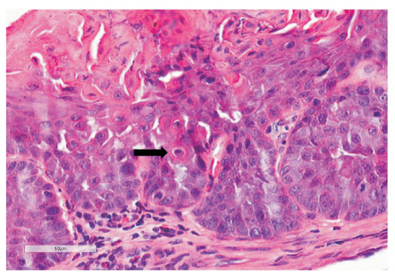
Summary of histological analysis of liver from male mice treated or not with NDEA 40 ppm.



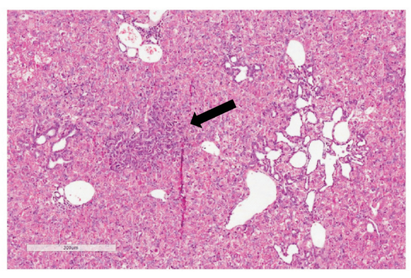
Morphological phenotype of esophageal moderate squamous dysplasia (arrow).



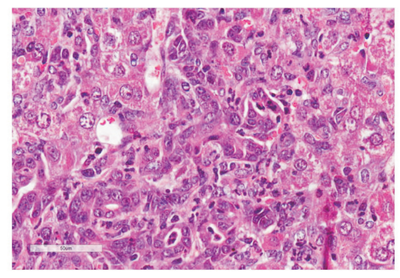
Morphological phenotype of an esophageal area with cellular atypia with (dyskeratosis - ellipse).



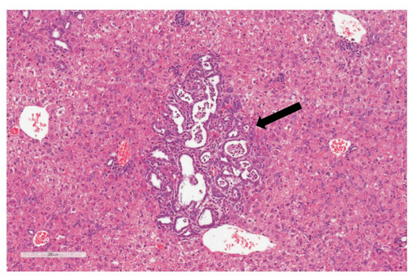
Morphological phenotype of an esophageal area of cellular atypia with dyskeratosis (rounded cells with eosinophilic cytoplasm). Dyskeratotic cell (arrow).



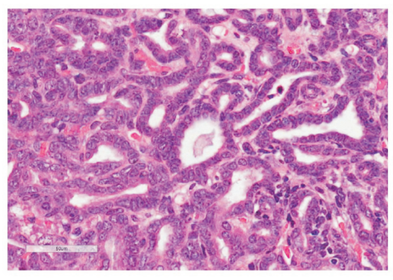
Morphological phenotype of the liver. Right side: area of proliferative tubule-glandular structures without atypia. Left side: area of invasive and proliferative tubule-glandular structures with high grade atypia (arrow).



Morphological phenotype of the liver. Infiltrative and proliferative tubule-glandular structures with high grade atypia. Hepathocellular carcinoma.



Morphological phenotype of the liver. Proliferative tubule-glandular structures with cellular atypia, without infiltrative appearance (arrow).



Morphological phenotype of the liver. Infiltrative and proliferative tubule-glandular structures with high grade atypia.

CONCLUSION

Low protein diet during pregnancy doesn't seem to affect the susceptibility of developing esophageal and liver tumors induced by NDEA in adult offspring.

REFERENCES

1. Jang, H; Serra,C (2014). Clin Nutr Res. 3:1-8.
2. Byrne CD; Phillips DI (2000). J Clin Pathol.53:822-8.
3. Ozanne SE (2001). Br Med Bull. 60:143-52.
4. Tollefsbol TO (2014). Transgenerational Epigenetics – Evidence and Debate. San Diego, USA: Elsevier, 396p.
5. Remacle C et al. (2011). Am J Clin Nutr. 94(6):1846S-1852S.
6. Lee HS and Herceg Z (2014). Cancer Letters. 342(2):275-84.
7. Painter, RC et al. (2006). Am J Hum Biol.18:853-856.
8. Fernandez-Twinn,et al. (2007). Carcinogenesis. 28(3):545-552.
9. Fernandez-Twinn et al. (2010).Carcinogenesis. 31(10):18:73-1881.

Funding source: Ministério da Saúde, FAPERJ

Animal Care and Use Committee of INCA – number: 032/13

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA

Ministério da Saúde

GOVERNO FEDERAL BRASIL PÁTRIA EDUCADORA