

动脉粥样硬化高低发区人冠状动脉内膜平滑肌细胞结蛋白及胸腺素表达的比较*

邓卓霖 赵培真^① 王爱忠^②

(广西医科大学病理学教研室, 南宁 530021)

Expression of Desmin and Prothymosin in Coronary Arterial Smooth Muscle Cells of Subjects from Districts of High and Low Incidence of Atherosclerosis

DENG Zhuo-Lin^①, ZHAO Pei-Zhen^② and WANG Ai-Zhong^③

(^①Guangxi Medical University, Nanning 530021; ^②Cardio-vascular Institute, Beijing 100037; ^③Ningbo 113 Hospital, Zhejiang 315200, China)

ABSTRACT Coronary arteries of youth from Beijing, Nanning and Ningbo were collected, taking ten cases from each district respectively. Desmin and prothymosin (PT) alpha in smooth muscle cells (SMC) of the intima of the coronary artery were detected using immunohistochemical method. The desmin positive rates in Nanning, Ningbo, and Beijing were in order of the frequency from high to low. By comparison the difference between Nanning and Beijing was dominant ($P=0.0004$) and the difference between Ningbo and Beijing was significant ($P=0.019$). While there was no statistical significance difference between Nanning and Ningbo ($P=0.2156$). It indicates that the SMC in the intima of the coronary artery are potentially differentiated into maturity in the youth of Nanning and Ningbo. However, in the youth of Beijing there is lack of thi potency. About the positive rates of PT, the order of frequency from high to low was Beijing, Nanning and Ningbo, though there was

^② 中国人民解放军第 113 医院病理科, 宁波 315200

no statistical significance. PT acted as proliferating cell nuclear antigen. It indicates that the SMC of the coronary arterial intima of young Beijinese persist in their proliferous potency. The above mentioned two results show that the SMC of the arterial intima in the districts of high and low incidence are different not only in the quantity of cells proliferation and secretion of heparan sulfate proteoglycan but, more importantly in their internal structures.

KEY WORDS Coronary atherosclerosis; Smooth muscle cell; Desmin; Prothymosin alpha

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摘要 用免疫组织化学方法研究北京、南宁和宁波青年人冠状动脉内膜平滑肌细胞中结蛋白和胸腺素的表达情况。测得结蛋白阳性率的高低顺序为南宁、宁波、北京。南宁与北京的差别有显著性($P=0.0004$), 宁波与北京的差别有意义($P=0.019$), 而南宁与宁波的差别无意义($P=0.2156$)。结蛋白可作为肌细胞分化的标志, 提示南宁与宁波青年冠状动脉内膜平滑肌细胞向成熟分化, 而北京人则缺乏这种倾向。前胸腺素阳性率顺序为北京、南宁、宁波。提示北京人冠状动脉内膜 SMC 相对有较高的增长趋势。上述结果显示高低发区 SMC 内部结构的差别。

关键词 冠状动脉粥样硬化; 平滑肌细胞; 结蛋白; 前胸腺素

我国动脉粥样硬化(atherosclerosis, As)发病率北方高, 南方低。我们前阶段的研究已发现北京与南宁是我国最高与最低发病地区之

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^① 阜外心血管病医院病理科, 北京 100037

—^[1]。南宁青年动脉内膜细胞增殖程度较北京低,而有抑制平滑肌细胞(smooth muscle cell, SMC)增殖作用的硫酸乙酰蛋白聚糖在动脉中的含量则较北京高^[1,2]。文献报道 SMC 的中间丝结蛋白(desmin, D)能反映细胞的成熟程度并与分泌功能有关^[3],而前胸腺素(psothymosin, PT)只见于增殖细胞而不见于静止(G₀)细胞^[4],国外报道高发区主动脉及冠状动脉只有中膜 SMC 结蛋白阳性,内膜则完全缺乏结蛋白阳性细胞^[5-8]。我们意外发现南宁人主动脉及冠状动脉内膜 SMC 中结蛋白阳性细胞数较多,冠状动脉更多于主动脉^[9]。SMC 中 PT 也呈阳性反应,估计与南宁人动脉内膜 SMC 对生长因子刺激不敏感有关。本文重新收集不同地区的病例作比较研究。

1 材料与方法

病例为南宁、北京及宁波青年居民中非正常死亡病例,每个地区已收集百余例作形态学对比研究。本研究仅采取同一时期(1993~1994)死亡的新鲜标本每地 10 例,年龄 18~30 岁,平均年龄为南宁 25.7 岁,北京 23 岁、宁波 25.4 岁。南宁与北京男性占 2/3,宁波男女各半。标本取冠状动脉左前降枝开口下方 1 cm 处,

避开了血管分支处,每例切一块,用酒精固定 24 h,石蜡包埋切片,HE 染色及 ABC 法作结蛋白及 PT 免疫组织化学染色。同一人同一批试剂按试剂说明书进行操作。HE 染色为避免有 As 斑块。

2 结果

2.1 结果的判定

内膜 SMC 免疫组织化学阳性反应呈棕黄色。结蛋白定位于胞浆中。PT 定位于细胞核中。两种反应除在细胞内分布不同外,在血管壁的地区分布亦略有不同。在内膜及中膜结蛋白阳性细胞分布均偏重外侧,而 PT 则无偏重外侧倾向,胞浆肥大者常阳性,而核瘦长者常阴性。结果见 Table 1。

2.2 结果的半定量分析

冠状动脉横切面面积只有 2 个低倍视野(100×),高倍视野(400×)10 个左右,极易观察。内膜结蛋白阳性细胞在北京的案例常一个也不见,因此能找见少数散在的结蛋白阳性细胞定为+(Figure 1),南宁的案例则内膜结蛋白阳性细胞较多,常占 SMC 总数的 25%左右定为++(Figure 2)。

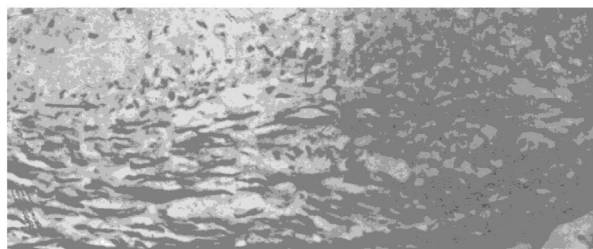


Figure 1. Coronary artery. The cells of the media are all desmin positive, while in the intima the positive cells are scattered distribution in the musculoelastic layer (short arrows). Long arrow shows the internal elastic lamina (IEL) between the intima and the media.

3 讨论

动脉粥样硬化的发生与发展都与内膜 SMC 增殖程度密切相关。正常成人中膜 SMC

为收缩表型环形排列于动脉壁,胞浆中有大量肌丝和致密斑,而 Golgi 体和粗面内质网则不发达。从出生开始动脉内膜 SMC 即缓慢增

Table. Compare the expression of desmin and prothymosin in SMC of intima of the coronary arteries from different area.

Nanning			Beijing			Ningbo		
No.	desmin	PT	No.	desmin	PT	No.	desmin	PT
Na166	++	-	B234	-	-	Ni69	+	-
Na167	++	-	B235	+	-	Ni72	+	+
Na168	++	-	B236	-	+	Ni74	+	+
Na169	++	-	B237	-	+	Ni77	-	-
Na170	++	-	B238	-	+	Ni79	-	-
Na171	++	+	B241	-	-	Ni80	+	-
Na172	++	+	B242	-	+	Ni81	+	-
Na173	++	+	B245	-	-	Ni82	+	-
Na174	++	-	B247	-	-	Ni83	+	-
Na175	++	-	B259	-	-	Ni73	-	-

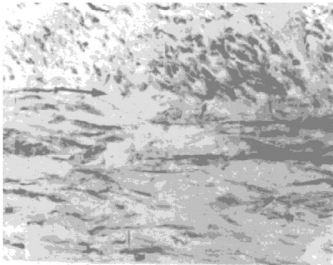


Figure 2. Lot of desmin positive SMC are observed in the musculoelastic layer of the intima (short arrows). The long arrow points on the IEL.

殖,至10步左右才形成完整的内膜肌弹力层。而冠状动脉内膜在出生时已有肌弹力层,以后随年龄和血压升高而增厚。内膜的厚度常超过中膜,说明内膜增生较快,其中主要是SMC增殖和产生基质。内膜的SMC通常为合成型,具有丰富的内质网和Golgi体,很少肌丝和致密斑,类似胚胎期和损伤后修复处的SMC,维持增殖活性和合成结缔组织基质,包括胶原、弹力纤维和蛋白聚糖,SMC及其合成的基质在内膜局部过量堆积是As的基础。胶原堆积的局部易发生裂伤或形成溃疡,蛋白聚糖能结合数种

分子,与脂质沉积、钙化和血栓形成有关。蛋白聚糖中也有能抑制SMC生长的硫酸乙酰肝素蛋白聚糖与SMC增殖程度及As形成呈负相关^[10,11]。

我们对比分析发现南宁人动脉壁硫酸乙酰肝素蛋白聚糖的含量明显高于北京人^[2]。电镜观察因视野过小,难于确定南宁人与北京人内膜SMC表型在数量上的差别。鉴于SMC中结蛋白阳性是其成熟的标志^[6-8],因此对比我国As发病率不同地区人冠状动脉SMC中结蛋白表达情况,对探讨病因学及发病学有重要意义,特别是30岁以前的青年人正是动脉内膜迅速增生时期,内膜增生主要以SMC为基础。南宁人冠状动脉内膜结蛋白阳性标本的百分率(100%)较北京人(10%)高,差别有显著性($P=0.0004$);宁波人(70%)与北京人差别有意义($P=0.019$);但南宁人与宁波人间的差别无意义($P=0.2156$)。南宁人阳性细胞的数量也较其他两地高。阳性例数多和每例阳性细胞数多说明南宁青年人冠状动脉内膜中的SMC趋向于成熟,与硫酸乙酰肝素蛋白聚糖含量成正相关。Nikkari^[12]和Sisto等^[13]发现内乳动脉内膜SMC的结蛋白含量多,该动脉很少发生As病变,用作冠状动脉旁路移植材料远期通畅率显著优于隐静脉,提示动脉内膜结蛋白阳性有抗

As 作用。

前阶段我们用图象分析发现北京人动脉内膜细胞密度和核密度均高于南宁人,主要是因 SMC 增殖。我们 PT 抗体免疫组织化学染色发现北京人冠状动脉内膜 PT 阳性率最高(50%),宁波人最低(20%),南宁人居间(30%)。PT 广泛分布于很多组织中,其作用类似增殖细胞核抗原,而更适合于福尔马林固定的标本研究。PT 仅见于增殖的细胞而不见于静止的细胞,在细胞周期中不见于 Go 期^[5]。北京人冠状动脉内膜 PT 阳性率高,提示其 SMC 相对处于活跃增殖状态,与结蛋白阳性和硫酸乙酰肝素蛋白聚糖含量呈负相关。过去未见有报道用 PT 测定动脉内膜 SMC,估计可用作 SMC 增殖的一个新指标。

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