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WORKSHOP REPORT

Advances in basic and clinical research in laminopathies

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Lamins (LMNA) are the main proteins of the nuclear lamina considered to be the ancestors of all intermediate filament proteins. They form complex protein assemblies with integral proteins of the inner nuclear membrane, transcriptional regulators, histones and chromatin modifiers. During recent years, interest in lamins has greatly increased due to the identification of many distinct heritable human disorders associated with lamin mutations. These disorders, collectively termed laminopathies, range from muscular dystrophies to premature aging. They may affect muscle, fat, bone, nerve and skin tissues. The workshop was addressed to understand lamin organization and its roles in nuclear processes, mutations in lamins affecting cell and tissues functions, the biology of the nucleus and laminopathic disease mechanisms, all aspects important for designing future therapies.

Key words: LMNA A/C gene, laminopathies, Emery-Dreifuss muscular dystrophy

A workshop dedicated to the advances in basic and clinical aspects of laminopathies was held in Warsaw, last 29-30th November 2012, organized by Irena Hausmanowa-Petrusewicz. The congress was scheduled as a two days format, the former dedicated to the advances in basic research, the latter to the advances in clinical research in the field of laminopathies.

Lamins (LMNA) are the main proteins of the nuclear lamina considered to be the ancestors of all intermediate filament proteins (1). They form complex protein assemblies with integral proteins of the inner nuclear membrane, transcriptional regulators, histones and chromatin modifiers. During recent years, interest in lamins has greatly increased due to the identification of many distinct heritable human disorders associated with lamin mutations. These disorders, collectively termed laminopathies, range from muscular dystrophies to premature aging. They may affect muscle, fat, bone, nerve and skin tissues. Understanding lamin organization, its roles in nuclear processes and why mutations in lamins affect cell and tissues functions is important for understanding the biology of the nucleus and laminopathic disease mechanisms, as far as for designing future therapies.

Effect of nuclear lamina and epigenetics in ageing mechanisms

Y. Gruenbaum showed the results obtained with his coworkers D.Z. Bar and M. Davidovich on the regulation of aging, by the C. elegans nuclear lamina. Lamins and most of their functions are conserved in Caenorhabditis elegans (2). Although linked to premature aging diseases, they have yet to be linked to any of the major lifespan regulating pathways, thus leaving a gap in the understanding of the lamins' role in natural aging. Dietary restriction (DR) acts via conserved pathways to enable better cell maintenance and prolongs lifespan and health-span in multiple organisms. In Caenorhabditis elegans, multiple aspects of DR are regulated by lamin, including animal length and fat content, in a pathway mediated by S6K and SREBP. Furthermore, some aspects of DR are regulated

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by specific changes in proteins at the nuclear envelope. C. Hutchison presented his studies on the role of lamin A in senescence in normal and premature ageing (3-5).

M. Puzianowska-Kuznicka reported the results obtained by her work group (M. Budzinska, M. Owczarz, E. Pawlik-Pachucka and J. Połosak) on epigenetics of immunosenescence. Aging results from accumulation of a stochastic damage to DNA, proteins, and to lipids. Its rate and clinical course depend on genetic, environmental, and stochastic factors. Studies performed on monozygotic twins (6) suggest that up to the age of 85, the rate of aging depends on genes only up to 35%, but the role of genetic factors increases thereafter. Genes potentially contributing to aging of humans are these encoding proteins involved in the insulin and insulin-like growth factor-1 (7) pathways, genes encoding sirtuins (8), lamin A/C, apolipoprotein E, enzymes de-activating the reactive oxygen species, and genes encoding proteins involved in DNA repair. Aging is accompanied by epigenetic drift, an age-related, tissue-specific change in the pattern of epigenetic modifications, that in a large part is a result of lifelong exposure to various environmental factors (9, 10). Age-related alterations of function of blood mononuclear cells might be, in part, a result of epigenetic drift affecting the level of expression of various genes. She showed that the expression of IGF-1R, FOXO1, FOXO3a, SIRT1-7, WRN, XPD, THRA and THRB genes significantly decreased with age (11, 12), in a different way.

Pathogenesis of laminopathies

The role of mesenchymal stem cells in the pathogenesis of Hutchincon-Gilford progeria syndrome was discussed by K. Domańska-Janik. Hutchinson-Gilford progeria syndrome (HGPS) is a sporadic genetic disease, extremely rare, linked with mutations of LMNA gene, presenting specific features of premature aging. A progressive deterioration of the various mesenchymal derived tissues was observed in laminopathies (13), leading in the past to hypothesize that the dysfunction of mesenchymal stem cells (MSCs) might be a specific target for mutation (14). Recent studies on the processes of maturation in the context of somatic stem cell biology have suggested that other hypotheses addressing the role of MSCs in the pathology of progeria would be equally plausible. Among them, the hypothesis of Melton and Cowan (15), which suggests that somatic stem cells residing in their tissue-specific niches are not necessarily part of a classical developmental continuum, but they may arise as a distinct pluripotent, embryonic-like stem cell lineage separated from the main stream of organogenesis (16). These cells could be grown in vitro for a long time as non-immortalized cell lines and differentiate also toward neurons and glia cells. She concluded that cultured lines of these stem cells could provide a valuable authologous material for transplantation to patients that present with progeria.

Role of lamins in chromatin organization

R. Foisner presented his studies aimed at clarifying the role of nucleoplasmic lamins in chromatin organization and possible implications for laminopathies (17). He has identified a nucleoplasmic A-type lamin-binding protein, termed Lamin-associated Polypeptide 2 alpha (LAP2α) (18), which impairs assembly of A-type lamins at the nuclear lamina and maintains a pool of soluble, mobile A-type lamins throughout the nucleus. He also showed that a nucleoplasmic complex of A-type lamins and LAP2α increases the repressor activity of the cell cycle regulatory retinoblastoma protein (pRb). Furthermore the deletion of the Lap2α gene in mice causes loss of nucleoplasmic lamins and a deregulation of pRb-mediated gene expression, leading to hyperproliferation of tissue progenitor cells and hyperplasia of the tissue (18). He proposed a model in which a nucleoplasmic pool of lamins is involved in the regulation of chromatin structure and function in tissue progenitor cells during tissue regeneration; he postulated that mutations in lamins can alter the ratio of nucleoplasmic versus peripheral lamins and thereby affect tissue progenitor cells and tissue regeneration.

Role of mutated lamin A and emerin proteins in development of abnormal phenotypes and prospects for gene therapy

This particular aspect of lamins was illustrated by R. Rzepecki. Mutations in LMNA and STA genes affect major cellular pathways regulating the development, maintenance and regeneration of tissues, mostly cardiac and skeletal muscles, of mesodermal origin. Lamin A, lamin B, emerin, NET25, NET39 and MAN1 (LEMD3) proteins modulate such signaling pathways e.g.: Wnt, TGFβ/BMP/activin, MAPKs, mTOR, Akt, PKC (19). Most of these pathways interconnect themselves and with many other pathways giving rise to the differences in manifestations of disease phenotypes. Preliminary reports demonstrate the possibility to use gene/cell therapy for the muscular dystrophy type of laminopathies as well as for HGPS Progeria. Strategies for gene therapy for AR type of laminopathies seem to be the simplest, while the prospect gene therapy treatment of AD laminopathies seems

to be much more complicated (20). Lentivirus vector system for delivery of genetic drug represents a model of universal gene therapy strategy for muscle laminopathies and HGPS progeria.

Clinical aspects of laminopathies

The second day was opened by G. Opolski who stressed the variety of LMNA clinical phenotypes, most of them with ***cardiac involvement,*** frequently characterized by arrhythmias and dilated cardiomyopathy (DCM). He presented a brief history of research in laminopathies within the field of cardiology, starting from the first description of a DCM case due to LMNA mutation (21), to case series of DCM with atrio-ventricular conduction defects, the natural history of LMNA DCM underlying the poor prognosis and the high risk of sudden cardiac death (SCD) in these patients. His series comprised 34 pts with genetically confirmed EDMD [24 pts with an X-linked inheritance (defect in the STA gene, emerinopathy) and 10 pts with an autosomal dominant form (defect in LMNA, laminopathy)], compared with 25 healthy volunteers. G. Opolski showed that cardiac involvement was independent of the severity of skeletal muscle disease, and that both left ventricular systolic (24%) and diastolic dysfunction (41%) are very common and responsible for a high risk of sudden death. Early detection of cardiac conduction disorders may be life-saving in pts with cardiomyopathy and LMNA mutation. He presented the guide-lines for the management of these patients, that follows the standards of treatment for heart failure and recommended ICD implantation also in patients requiring pacing who do not meet generally accepted criteria for ICD in the general population (22-24).

L. Politano presented a combined talk dealing with the wide spectrum of myo-cardiolaminopathies in humans, and the treatment of arrhythmic events in laminopathies, in collaboration with Gerardo Nigro. Different clinical presentations associated with mutations in LMNA gene, ranging from classical AD-EDMD phenotype involving both skeletal muscles and myocardium (25, 26), to LGMD phenotype (27), "pure" cardiac presentation as brady-arrhythmias (sino-atrial or atrio-ventricular blocks of several degree) or tachy-arrhythmias (atrial or ventricular fibrillation or flutter, ventricular tachycardia) without any skeletal muscle involvement (28) were shown. Particular emphasis was done on the congenital phenotype of laminopathies, presenting as a congenital muscular dystrophy (29) but associated with a high frequency of arrhythmias and risk of SCD. Future therapeutic possibilities arising from drugs enhancing autophagy such as temsirolimus, or from MTOR blockade (30), were presented on the basis of a defective autophagy in hearts of *LMNA* mutated (H222P/H222P) mice recently reported (31).

The usefulness of not invasive elettrocardiographic parameters such as QTc dispersion (QTc-D), JTc dispersion (JTc-D) and Tpeak-end dispersion (TDR), that reflect the physiological variability of regional and transmural ventricular repolarisation and provide a substrate for life-threatening ventricular arrhythmias was also stressed. In the experience of the Naples group, EDMD is associated with increased heterogeneity of ventricular repolarisation even in the absence of impaired systolic and diastolic cardiac function (32-33).

The last two lectures were dedicated to the description of LMNA prevalence in two different realities: the Sardinia isle in Italy and the Poland country.

N. Carboni showed his database including 46 subjects with LMNA gene mutations, all but 1 familial cases. He presented one of the families showing familial dilated cardiomyopathy with conduction defects due to mutation in Lamin A/C gene (28). Patients with overlapping syndromes, obtained by the concomitant presence of cardiac compromise, late lipodystrophy of the Dunnigan type, diabetes and axonal neuropathy (34) and a series of pictures of lower limbs muscle MRI were shown. Despite the different (prevalently cardiac or muscle) phenotype, all patients had a similar pattern of posterior leg's muscles involvement, affecting medial head of gastrocnemius, sartorius and lateral head of gastrocnemius (35). Follow up studies on larger cohorts of patients are to be encouraged and the experience of the Italian Centre for Laminopathies taken as an example of a fruitful collaboration (36, 37).

Irena Hausmanowa-Petrusewicz concluded the congress reporting *various aspects of laminopathies in Poland*. She said: "Our adventure with laminopathies started long time ago when we, by chance, got for consultation the patient whom we were unable to recognize as were also same with local doctors. The diagnosis in this patient was made by British colleagues, who recognized laminopathy, which was a terminology unknown to us. In spite of this we began fascinated by this problem. We started and still are working on laminopathies (38, 39). The historic patient was a member of huge family P., affected by emerinopathy (mutation in EMD gene). We had access many members of this family. The patients were only males, and we checked carriers, who were mostly fifty or sixty year old females, developing at this age cardiac symptoms. Such cardiac symptoms became clear to us as a part of clinical picture, following muscle involvement and joint contractures. Quite soon after identification of the second gene associated with similar clinical presentation we found also in Poland many cases which had the same phenotype, resulting from muta-

tions in another gene, LMNA, encoding lamin A/C. The most fascinating problem became to us the striking variability (inter- and intrafamiliar) of phenotype in laminopathic disorders. Our clinical activity was concentrated on therapy, provided by the Department of cardiology, chaired by prof. Opolski (39). In the following years we started to look for patients in the clinical centers of our country and as a result we became still modest, but anyway leading center of laminopathies in Poland. We recognized better the pathology of nuclear proteins i.a. that expressed in other tissues, manifesting as lipodystrophy, peripheral neuropathy, isolated cardiomyopathy and progeria. In the meantime our colleagues became interested in some specific problems in laminopathies: Niebrój-Dobosz – in biomarkers (40-42), which turned out to be important for diagnosis and prognosis in cardiac involvement; Fidziańska – in ultrastructural analysis of affected myocytes indicating characteristic structural changes of nuclei (43). The last issue till now, which arose our interest were laminopathies in children, i.e. congenital dystrophy, restrictive dermopathy and progeria, which lead us to problem of premature aging. Madej-Pilarczyk described a large family affected by overlapping syndrome of progeria and restrictive dermopathy, associated with homozygous mutation in LMNA gene (44). Our next step would be continuation of present work with special attention on the role of laminopathies in development and in normal and premature aging".

Conclusions

Fruitful discussion during all the meeting clarified different points of view, and constructively resulted in a proposal for a wide European collaboration. The interdisciplinary approach to laminopathies was highly encouraged. This was an enjoyable and fruitful workshop that will lead to new collaborations and will contribute significantly to the improvement of future therapeutic perspectives in laminopathies.

List of participants

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