

Research Article – Histology and Cell Biology

A role for apoptosis in temporomandibular joint disc degeneration. A contemporary review

Concetta Galanti^{1*}, Giuseppe Musumeci², Jessica Valentino¹, Salvatore Giunta², Sergio Castorina²¹ Department of Dentistry, Faculty of Dentistry, University of Catania, Policlinico Universitario, Catania, Italy² Department of Bio-Medical Sciences, Section of Anatomy, University of Catania, Catania, Italy

Submitted October 19, 2012; accepted October 28, 2012

Summary

The temporomandibular joint (TMJ) connects the mandible to the skull. TMJ disorders induce degenerative tissue changes in TMJ disc that are largely the result of maladaptation to abnormal joint loading. Histopathological studies have documented an association between TMJ arthropathy and loss of tissue cellularity, via apoptosis-related processes, that result in diminished extracellular matrix generation, organization, and repair. However, the exact molecular mechanisms underpinning the development and progression of such degenerative changes are still unclear. We review the most recent findings regarding the involvement of apoptotic mechanisms in TMJ disc degeneration. Although a number of aspects of TMJ disc degeneration have been thoroughly investigated, data on the involvement of apoptotic mechanisms and their mediators are few and quite recent; indeed most of the research conducted on fibrous cartilage apoptosis has focused on the intervertebral disc.

Key words

Fibrocartilage; abnormal loading; disc displacement; cell phenotype.

Introduction

The temporomandibular joint (TMJ) connects the mandible to the skull (Panmekiate et al., 1991; Piette, 1993; Tong and Tideman, 2001). It is a synovial joint that allows jaw opening and closing and forward, backward and lateral excursion, i.e. the movements that make it possible to bite, chew, swallow, speak, and make facial expressions. The unique feature of the TMJ is the disc separating the articulating surface of the glenoid fossa and eminence from that of the mandibular condyle and dividing the joint cavity into two compartments (Kapila et al., 1995). TMJ disorders have been demonstrated to induce degenerative disc tissue changes that are largely the result of maladaptation to abnormal joint loading (Haskin et al., 1995; Kirk, 1990; Leonardi et al., 2007; Loreto et al., 2009). Histopathological investigations of degenerated TMJ disc have documented an association between TMJ arthropathy and loss of tissue cellularity via apoptosis-related processes that lead to diminished extracellular matrix (ECM) generation, organization, and repair (Leonardi et al., 2002, 2008, 2010; Loreto et al., 2010; Peagle et al., 2003; Hamada et al., 2008; Matsumoto et al., 2008). Although collagen bundle

*Corresponding author. E-mail: cetin@hotmail.it; Tel: +39 0953 782475; Fax: +39 0953 782475.

fragmentation, tearing and splitting, disc perforation, cell loss and non-specific degenerative changes have been extensively described, the molecular mechanisms underpinning their development and progression are still unclear (Kang et al., 2006).

Disc structure and degeneration mechanisms

Histologically the TMJ disc is a compact aneural and nearly avascular fibrous structure (Marchetti et al., 1999; Leonardi et al., 2001) characterized by distinctive fibrocartilage cells in an ECM containing clearly visible collagen and/or elastic fibres resembling ordinary dense fibrous connective tissue. Its mechanical properties rely on ECM composition and are ensured by three cell populations: elongated or fibroblast-like cells, the most numerous; rounded cells without a pericellular halo (fibrochondrocytes), and rounded cells with a pericellular halo (chondrocyte-like cells) (Miliam et al., 1991; Berkovitz and Pacy, 2000; Detamore and Athanasiou, 2003). Fibrocartilage is a dynamic tissue that responds to changes in mechanical stress (Loreto et al., 2009). It has been demonstrated that maintenance of this phenotype requires application of intermittent compression and/or shear forces (Benjamin and Ralphs, 2004). Immunohistochemically fibrocartilage is characterized by type II collagen, aggrecan, laminin, elastin, and several other molecules such as aquaporin 1 and lubricin, whose expression is regulated by tissue homeostasis mechanisms (Leonardi et al., 2012a, b; Loreto et al., 2012) and is altered in degenerated tissue (Leonardi et al., 2008, 2011; Loreto et al., 2012).

Histologically disc displacement is associated with degenerative changes that induce an active cell response and a phenotype change from fibroblast-like to fibrochondrocyte and eventually to chondrocyte-like (Loreto et al., 2009). TMJ discs affected by internal derangement, which involves altered anatomical relationships of the disc-condyle complex, exhibit a general tissue remodelling induced by abnormal loading (Leonardi et al. 2007). Leonardi et al. (2010) recently devised a histopathological score involving a semiquantitative transcription of the full spectrum of TMJ disc degenerative diseases depending on changes in disc tissue. However, degenerative changes are also a function of the type and degree of disc displacement; advanced internal derangement corresponds to a severely deformed disc.

The adult human TMJ disc contains blood vessels only in the bilaminar zone. The poor blood supply and the compact arrangement of the fibrous component are held to be the morphological characteristics underpinning the disc's ability to withstand the compression forces associated with articulation (de Bont et al., 1985). In contrast, a dense vascularity is found in discs affected by internal derangement, which often leads to histopathological changes that culminate in tissue degeneration (Marchetti et al., 1995; Yoshida et al., 1999).

Apoptosis in TMJ disc with internal derangement

Apoptosis, or programmed cell death, is a multi-step, evolutionarily conserved homeostatic mechanism by which cells undergo an orderly demise. Its role is well known in human neoplastic and non-neoplastic disease (Zamparese et al., 2008; De Maria et al., 2009; Leonardi et al., 2012 in press; Loreto et al., 2012, in press; Ouy-

ang et al., in press). At variance with necrosis, the contents of the apoptotic cell are not released and the cell is cleared by phagocytosis (Sedlakova et al., 1999; Malmusi and Ackerman, 2000; Kanduc et al., 2002; Power et al. 2002; Elmore et al., 2007; Rezzani et al., 2012). Apoptosis works through two main, alternative pathways: death receptor-mediated (or extrinsic) and mitochondria-dependent (or intrinsic). The former pathway is initiated by ligation of specific death receptors by their ligands. The main death receptors—Fas and tumour necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) receptors R1 and R2—induce cell death following ligation with Fas ligand (FasL) or TRAIL, respectively (Rodella et al., 2010; Leonardi et al., 2011; Loreto et al., 2011). Ligation of TRAIL R1 by TNF α also induces apoptosis after inhibition of nuclear factor kappa-B (NF- κ B). Fas ligation by FasL is followed by recruitment of FADD (Fas-associated via death domain) and subsequently of caspase 8. This process gives rise to caspase 8 activation, which can be inhibited by the anti-apoptotic molecule FLICE inhibitory protein (Flip). Caspase 8 induces apoptosis by directly activating caspase 3 or by cleaving bid (BH3 interacting domain death agonist), resulting in mitochondrial dysfunction and subsequent release of cytochrome c and activation of caspases 9 and 3. Caspase 3 promotes the typical apoptosis features, including DNA fragmentation and cell death in several tissues (; Malmusi and Ackerman, 2000; Kanduc et al., 2002; Power et al. 2002; Elmore et al., 2007; Loreto et al., 2011; Rezzani et al., 2012).

It is unclear whether repetitive loading and apoptosis are quantitatively related in any type of cartilage. However, apoptosis has been documented to have a central role not only in intervertebral disc degeneration (Ariga et al., 2001; Park et al., 2001; Bertram et al., 2009; Loreto et al., 2011). Several studies have demonstrated the crucial function of apoptotic mechanisms in intervertebral disc degeneration, and the involvement of apoptosis in various conditions associated with intervertebral disc degeneration has been thoroughly explored (Rannou et al., 2004; Heyde et al., 2006; Hiyama et al., 2008; Tschoeke et al., 2008; Yang et al., 2008; Loreto et al., 2011; Caltabiano et al., in press).

Although a number of aspects of TMJ disc degeneration have been thoroughly investigated, data on the involvement of apoptotic mechanisms and their mediators are few and quite recent; indeed most of the research conducted on disc apoptosis has focused on the intervertebral disc. TMJ disc degeneration is believed to be a consequence of mechanical and biological events affecting the equilibrium between matrix synthesis and degradation. According to this hypothesis loss of cellularity, collagen fibre fragmentation and TMJ disc tears and clefts would all be underpinned by hyperapoptosis, a situation where collagen fibre degradation is not offset by synthesis of new fibres because of apoptosis-induced cell loss. In turn, a protracted imbalance between matrix synthesis and degradation leads to increasingly severe TMJ disc defects such as clefts and tears. The findings correlating TMJ disc internal derangement and apoptosis largely come from animal models (Matsuda et al., 1997; Gu et al., 2002; Nagai et al., 2003; Spears et al., 2003; Huang et al., 2004). However, the Italian group headed by Leonardi has extensively analyzed programmed cell death in human TMJ disc with and without internal derangement (Loreto et al 2011a). These authors have advanced the hypothesis that the degenerative changes seen in discs with internal derangement must include an active cell response with a change in cell phenotype from fibroblast-like to fibrochondrocyte, and eventually to chondrocyte-like, possibly as a response to abnormal loading. However, since only small areas of

chondral tissue are usually detected in affected discs, they postulated the involvement of apoptotic processes to explain the incomplete tissue change. They did find activation of apoptosis via the extrinsic and the intrinsic pathway, and noted that the degree of apoptosis activation correlated with the degree of disc degeneration. After Huang and co-workers demonstrated that Bcl-2 and Bax oncoproteins are physiologically expressed in rabbit craniomandibular joint, Leonardi's group investigated the activation of the intrinsic pathway and demonstrated an involvement of these molecules in human TMJ disc with internal derangement (Caltabiano et al., in press). In fact the mitochondrial pathway is partly influenced by Bcl family members bound to the mitochondrial membrane, including Bax and Bcl-2, which are respectively pro- or anti-apoptotic regulatory proteins. The anti-apoptotic proteins Bcl-2 and Bcl-XL inhibit cytochrome c release, whereas Bcl-2-associated X protein (Bax), Bcl-2 homologous antagonist/killer (Bak), and Bid, all pro-apoptotic proteins, promote its release from mitochondria. Cytochrome c and deoxyadenosine triphosphate (dATP) bind to apoptotic protease activating factor (Apaf-1) to form a multimeric complex that recruits and activates procaspase 9, an apoptosis-mediating executioner protease that in turn activates caspase 3, resulting in cell apoptosis.

Further research subsequently led the Italian group to document the presence of vessel apoptosis in TMJ discs with internal derangement (Loreto et al., 2010). The findings generated the hypothesis that apoptosis activation could be a self-limiting process directed at reversing the angiogenesis typically seen in these discs, *i.e.* a strategy aimed at limiting pathological angiogenesis by reducing the blood supply to tumours or by reducing harmful inflammation (Loreto et al., 2010). The strong immunorexpression of TRAIL, its receptor DR5 and caspase 3 documented in the intima and media layers of newly formed vessels seems indeed to reflect the defensive activation of the apoptosis process (Leonardi et al., 2010).

Conclusions

A therapeutic approach aimed at neutralizing apoptosis-inducing molecules would at least help delay the progression of disc degeneration (Alenzi, 2005). Identification of target molecules for gene construct or biological or chemical reagent delivery to target sites could contribute to prevent TMJ disc degeneration (Elmore, 2007).

References

- Alenzi F.Q. Apoptosis and diseases: regulation and clinical relevance. (2005) Saudi Med. J. 26: 1679-1690.
- Ariga K., Miyamoto S., Nakase T., Okuda S., Meng W., Yonenobu K., Yoshikawa H. (2001) The relationship between apoptosis of endplate chondrocytes and aging and degeneration of the intervertebral disc. Spine 26: 2414-2420.
- Benjamin M., Ralphs J.R. (2004) Biology of fibrocartilage cells. Int. Rev. Cytol. 233: 1-45.
- Berkovitz B.K., Pacy J. (2000) Age changes in the cells of the intra-articular disc of the temporomandibular joints of rats and marmosets. Arch. Oral Biol. 45: 987-995.

- Bertram H., Nerlich A., Omlor G., Geiger F., Zimmermann G., Fellenberg J. (2009) Expression of TRAIL and the death receptors DR4 and DR5 correlates with progression of degeneration in human intervertebral disks. *Mod. Pathol.* 22: 895-905.
- Caltabiano R., Leonardi R., Musumeci G., Bartoloni G., Rusu M.C., Almeida L.E., Loreto C. (in press) Apoptosis in temporomandibular joint disc with internal derangement involves mitochondrial-dependent pathways. An in vivo study. *Acta Odontol. Scand.*
- de Bont L.G., Liem R.S., Havinga P., Boering G. (1985) Fibrous component of the temporomandibular joint disk. *Cranio* 3: 368-373.
- De Maria S., Pannone G., Bufo P., Santoro A., Serpico R., Metafora S., Rubini C., Pasquali D., Papagerakis S.M., Staibano S., De Rosa G., Farina E., Emanuelli M., Santarelli A., Mariggì M.A., Lo Russo L., Lo Muzio L. (2009) Survivin gene-expression and splicing isoforms in oral squamous cell carcinoma. *J. Cancer Res. Clin. Oncol.* 135: 107-116.
- Detamore M.S., Athanasiou K.A. (2003) Structure and function of the temporomandibular joint disc: implications for tissue engineering. *J. Oral Maxillofac. Surg.* 61: 494-506.
- Elmore S. (2007) Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35: 495-516.
- Gu Z., Shibata T., Cao Z., Feng J., Hu J. (2002) Chondrocyte apoptosis in temporomandibular joints with disc displacement. *J. Oral Maxillofac. Surg.* 60: 1026-1031.
- Hamada Y., Kondoh T., Holmlund A.B., Sakota K., Nomura Y., Seto K. (2008) Cytokine and clinical predictors for treatment outcome of visually guided temporomandibular joint irrigation in patients with chronic closed lock. *J. Oral Maxillofac. Surg.* 66: 29-34.
- Haskin C.L., Milam S.B., Cameron I.L. (1995) Pathogenesis of degenerative joint disease in the human temporomandibular joint. *Crit. Rev. Oral Biol. Med.* 6: 248-277.
- Heyde C.E., Tschöcke S.K., Hellmuth M., Hostmann A., Ertel W., Oberholzer A. (2006) Trauma induces apoptosis in human thoracolumbar intervertebral discs. *BMC Clin. Pathol.* 6: 5.
- Hiyama A., Mochida J., Iwashina T., Omi H., Watanabe T., Serigano K., Tamura F., Sakai D. (2008) Transplantation of mesenchymal stem cells in a canine disc degeneration model. *J. Orthop. Res.* 26: 589-600.
- Huang Q., Singh B., Sharawy M. (2004) Immunohistochemical analysis of Bcl-2 and Bax oncoproteins in rabbit craniomandibular joint. *Arch. Oral Biol.* 49: 143-148.
- Kanduc D., Mittelman A., Serpico R., Sinigaglia E., Sinha A.A., Natale C., Santacroce R., Di Corcia M.G., Lucchese A., Dini L., Pani P., Santacroce S., Simone S., Bucci R., Farber E. (2002) Cell death: apoptosis versus necrosis (review). *Int. J. Oncol.* 21: 165-170.
- Kang H., Bao G.J., Qi S.N. (2006) Biomechanical responses of human temporomandibular joint disc under tension and compression. *Int J. Oral Maxillofac. Surg.* 35: 817-821.
- Kapila S., Lee C., Richards D.W. (1995) Characterization and identification of proteinases and proteinase inhibitors synthesized by temporomandibular joint disc cells. *J. Dent. Res.* 74: 1328-1336.
- Kirk Jr W.S. (1990) Morphologic differences between superior and inferior disc surfaces in chronic internal derangement of the temporomandibular joint. *J. Oral Maxillofac. Surg.* 48: 455-460.

- Leonardi R., Villari L., Bernasconi G., Caltabiano M. (2001) Histochemical study of the elastic fibers in pathologic human temporomandibular joint discs. *J. Oral Maxillofac. Surg.* 59: 1186-1192.
- Leonardi R., Caltabiano M., Cascone P., Loreto C. (2002) Expression of heat shock protein 27 (HSP27) in human temporomandibular joint discs of patients with internal derangement. *J. Craniofac. Surg.* 13:713-717.
- Leonardi R., Loreto C., Barbato E., Polimeni A., Caltabiano R., Lo Muzio L. (2007) A histochemical survey of the human temporomandibular joint disc of patients with internal derangement without reduction. *J. Craniofac. Surg.* 18: 1429-1433.
- Leonardi R., Loreto C., Barbato E., Caltabiano R., Lombardo C., Musumeci G., Lo Muzio L. (2008) MMP-13 (collagenase 3) localization in human temporomandibular joint discs with internal derangement. *Acta Histochem.* 110: 314-318.
- Leonardi R., Almeida L.E., Trevilatto P.C., Loreto C. (2010a) Occurrence and regional distribution of TRAIL and DR5 on temporomandibular joint discs: comparison of disc derangement with and without reduction. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 109: 244-251.
- Leonardi R., Rusu M.C., Loreto C. (2010b) Temporomandibular joint disc: a proposed histopathological degeneration grading score system. *Histol. Histopathol.* 25: 1117-1122.
- Leonardi R., Almeida L.E., Loreto C. (2011a) Lubricin immunohistochemical expression in human temporomandibular joint disc with internal derangement. *J. Oral Pathol. Med.* 40: 587-592.
- Leonardi R., Almeida L.E., Rusu M., Sicurezza E., Palazzo G., Loreto C. (2011b) Tumor necrosis factor-related apoptosis-inducing ligand expression correlates to temporomandibular joint disk degeneration. *J. Craniofac. Surg.* 22: 504-508.
- Leonardi R., Musumeci G., Sicurezza E., Loreto C. (2012a) Lubricin in human temporomandibular joint disc: an immunohistochemical study. *Arch. Oral Biol.* 57: 614-619.
- Leonardi R., Rusu M.C., Loreto F., Loreto C., Musumeci G. (2012b) Immunolocalization and expression of lubricin in the bilaminar zone of the human temporomandibular joint disc. *Acta Histochem.* 114: 1-5.
- Leonardi R., Matthews J.B., Loreto C., Musumeci G., Campisi G., Lo Muzio L., dos Santos J.W., Pastorino L., Bufo P., Pannone G. (in press) Beta-catenin and survivin expression in Keratocystic Odontogenic Tumor (KCOT). A comparative immunohistochemical study in primary, recurrent and nevoid basal cell carcinoma syndrome (NBCCS)-associated lesion. *Histol. Histopathol.*
- Loreto C., Musumeci G., Leonardi R. (2009) Chondrocyte-like apoptosis in temporomandibular joint disc internal derangement as a repair-limiting mechanism. An in vivo study. *Histol. Histopathol.* 24: 293-298.
- Loreto C., Almeida L.E., Migliore M.R., Caltabiano M., Leonardi R. (2010) TRAIL, DR5 and caspase 3-dependent apoptosis in vessels of diseased human temporomandibular joint disc. An immunohistochemical study. *Eur. J. Histochem.* 54: e40.
- Loreto C., Almeida L.E., Trevilatto P. (2011a) Apoptosis in displaced temporomandibular joint disc with and without reduction: an immunohistochemical study. *J. Oral Pathol. Med.* 40: 103-110.
- Loreto C., Barbagli G., Djinovic R., Vespasiani G., Carnazza M.L., Miano R., Musumeci G., Sansalone S. (2011b) Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its death receptor (DR5) in Peyronie's disease. A biomolecular study of apoptosis activation. *J. Sex. Med.* 8: 109-115.

- Loreto C., Musumeci G., Castorina A., Loreto C., Martinez G. (2011c) Degenerative disc disease of herniated intervertebral discs is associated with extracellular matrix remodeling, vimentin-positive cells and cell death. *Ann. of Anat.* 193: 156-162.
- Loreto C., Galanti C., Almeida L.E., Leonardi R., Pannone G., Musumeci G., Carnazza M.L., Caltabiano R. (2012a) Expression and localization of aquaporin-1 in temporomandibular joint disc with internal derangement. *J. Oral Pathol. Med.* 41: 642-647.
- Loreto C., Lo Castro E., Musumeci G., Loreto F., Rapisarda G., Rezzani R., Castorina S., Leonardi R., Rusu M.C. (2012b) Aquaporin 1 expression in human temporomandibular disc. *Acta Histochem.* 114: 744-748.
- Loreto C., Galanti C., Leonardi R., Musumeci G., Pannone G., Palazzo G., Rusu M.C. (in press) Possible role of apoptosis in the pathogenesis and clinical evolution of radicular cyst. An immunohistochemical study. *Int. Endod. J.*
- Malmusi M., Ackerman A.B. (2000) A critical review of apoptosis in historical perspective. *Am. J. Dermatopathol.* 22: 291-293.
- Marchetti C., Cornaglia I., Casasco A., Bernasconi G., Baciliero U., Stetler-Stevenson W.G. (1999) Immunolocalization of gelatinase-A (matrix metalloproteinase-2) in damaged human temporomandibular joint discs. *Arch. Oral Biol.* 44: 297-304.
- Matsuda S., Mishima K., Yoshimura Y., Hatta T., Otani H. (1997) Apoptosis in the development of the temporomandibular joint. *Anat. Embryol. (Berl.)* 196: 383-391.
- Matsumoto T., Tojyo I., Kiga N., Hiraishi Y., Fujita S. (2008) Expression of ADAMTS-5 in deformed human temporomandibular joint discs. *Histol. Histopathol.* 23: 1485-1493.
- Milam S.B., Klebe R.J., Triplett R.G., Herbert D. (1991) Characterization of the extracellular matrix of the primate temporomandibular joint. *J. Oral Maxillofac. Surg.* 49: 381-391.
- Nagai H., Kumamoto H., Fukuda M., Takahashi T. (2003) Inducible nitric oxide synthase and apoptosis-related factors in the synovial tissues of temporomandibular joints with internal derangement and osteoarthritis. *J. Oral Maxillofac. Surg.* 61: 801-807.
- Ouyang L., Shi Z., Zhao S., Wang F.T., Zhou T.T., Liu B., Bao J.K. (in press) Programmed cell death pathways in cancer: a review of apoptosis, autophagy and programmed necrosis. *Cell Prolif.*
- Paegle D.I., Holmlund A.B., Hjerpe A. (2003) Matrix glycosaminoglycans in the temporomandibular joint in patients with painful clicking and chronic closed lock. *Int. J. Oral Maxillofac. Surg.* 32: 397-400.
- Panmekiate S., Petersson A., Akerman S. (1991) Some anatomical factors of the upper compartment of the temporomandibular joint related to the disc position. *Int. J. Oral Maxillofac. Surg.* 20: 375-377.
- Park J.B., Kim K.W., Han C.W., Chang H. (2001) Expression of Fas receptor on disc cells in herniated lumbar disc tissue. *Spine* 26: 142-146.
- Piette E. (1993) Anatomy of the human temporomandibular joint. An updated comprehensive review. *Acta Stomatol. Belg.* 90:103-127.
- Power C., Fanning N., Redmond H.P. (2002) Cellular apoptosis and organ injury in sepsis: a review. *Shock* 18: 197-211.
- Rannou F., Lee T.S., Zhou R.H., Chin J., Lotz J.C., Mayoux-Benhamou M.A., Barbet J.P., Chevrot A., Shyy J.Y. (2004) Intervertebral disc degeneration: the role of the mitochondrial pathway in annulus fibrosus cell apoptosis induced by overload. *Am. J. Pathol.* 164: 915-924.

- Rezzani R., Stacchiotti A., Rodella L.F. (2012) Morphological and biochemical studies on aging and autophagy. *Ageing Res. Rev.* 11: 10-31.
- Rodella L.F., Favero G., Boninsegna R., Borgonovo A., Rezzani R., Santoro F. (2010) TGF-beta1 and VEGF after fresh frozen bone allograft insertion in oral-maxillo-facial surgery. *Histol. Histopathol.* 25: 463-471.
- Spears R., Oakes R., Bellinger L.L., Hutchins B. (2003) Tumour necrosis factor-alpha and apoptosis in the rat temporomandibular joint. *Arch. Oral Biol.* 48: 825-834.
- Tong A.C., Tideman H. (2001). The microanatomy of the rhesus monkey temporomandibular joint. *J. Oral Maxillofac. Surg.* 59: 46-52.
- Tschoeke S.K., Hellmuth M., Hostmann A., Robinson Y., Ertel W., Oberholzer A., Heyde C.E. (2008) Apoptosis of human intervertebral discs after trauma compares to degenerated discs involving both receptor-mediated and mitochondrial-dependent pathways. *J. Orthop. Res.* 26: 999-1006.
- Yang S.H., Wu C.C., Shih T.T., Chen P.Q., Lin F.H. (2008) Three-dimensional culture of human nucleus pulposus cells in fibrin clot: comparisons on cellular proliferation and matrix synthesis with cells in alginate. *Artif. Organs* 32: 70-73.
- Yoshida H., Fujita S., Nishida M., Iizuka T. (1999) Angiogenesis in the human temporomandibular joint studied by immunohistochemistry for CD34 antigen. *J. Oral Pathol. Med.* 28: 289-292.
- Zamparese R., Pannone G., Santoro A., Lo Muzio L., Corsi F., Pedicillo M.C., Scillitani E.L., Tortorella S., Staibano S., Piscuoglio S., Lo Russo L., Bufo P. (2008) Survivin expression in renal cell carcinoma. *Cancer Invest.* 26: 929-935.