Intratumoral versus Peritumoral Microvessel Density Scores in Ameloblastoma Subtypes

CHONG HUAT SIAR,*[1] ISMADI ISHAK [1] and KOK HAN NG,[2]

[1]Faculty of Dentistry, Univ. of Malaya, and [2]formerly Institute for Med. Research, Kuala Lumpur, MALAYSIA.

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STATEMENT OF THE PROBLEM:

Ameloblastomas constitute a clinically important group of tumours of the human jawbones. Although classified as benign, they have a locally-invasive behaviour and a tendency to recur after treatment. To date, the molecular mechanism(s) underlying this invasiveness and recurrence potential is ill-understood. As angiogenesis are known to influence tumoral growth, clarification of the microvessel density scores would shed light on their biological impact in this neoplasm.

PURPOSE:

To determine and compare intratumoral and peritumoral MVD scores in different ameloblastoma subtypes, and to evaluate their impact on the biological behavior of these neoplasms.

KEY FINDINGS:

- MVD score was significantly higher in SMA compared to UA. ($p<0.05$)
- MVD score was significantly higher in recurrent compared to primary ameloblastoma.
- Intratumoral and peritumoral MVD scores were not significantly different within each subtype

CONCLUSIONS:

- tumor angiogenesis is upregulated in ameloblastoma subtype with a clinically aggressive course

Table 3. Immunohistochemical results

Ameloblastoma subtypes	Total		CD34			CD31			CD105		
	n	%	Overall	Peri-T	Intra-T	Overall	Peri-T	Intra-T	Overall	Peri-T	Intra-T
Plexiform (P)	16	19.8	71.65	64.73	77.71	85.65	86.31	84.91	22.31	20.21	24.29
Follicular (F)	3	3.7	71.07	72.73	69.40	76.5	81.87	71.13	12.36	14.40	11.00
P+F	5	6.2	55.43	58.28	52.58	49.97	52.65	47.27	5.98	4.08	7.88
Granular cell	3	3.7	77.12	63.23	91.00	67.12	36.30	87.67	1.63	0	3.37
Acanthomatous	3	3.7	93.29	70.18	116.4	106.5	99.52	113.4	34.63	34.07	35.02
Basaloid	2	2.5	84.85	83.20	86.50	47.60	55.5	39.70	30.85	28.90	32.80
Unicystic	26	32.1	56.25	55.23	58.89	53.54	55.60	51.03	15.97	14.91	17.07
Desmoplastic	3	3.7	56.97	56.00	57.93	38.78	32.57	45.00	0	0	0
Peripheral	4	4.8	85.33	72.60	91.70	95.64	97.70	94.27	68.3	47.80	75.13
Recurrent	16	19.8	77.45	84.94	71.36	74.17	74.23	74.13	8.52	7.36	11.54

MATERIALS & METHODS:

Source:
Test samples were from the Oral Pathology Diagnostic and Research Laboratory, Faculty of Dentistry, University Malaya, Kuala Lumpur, Malaysia.

Materials:
Eighty-one ameloblastoma cases based on WHO criteria were selected and patient data retrieved (Tabel 1).
Antibodies to CD34, CD31 and CD105 were obtained (Table 2).

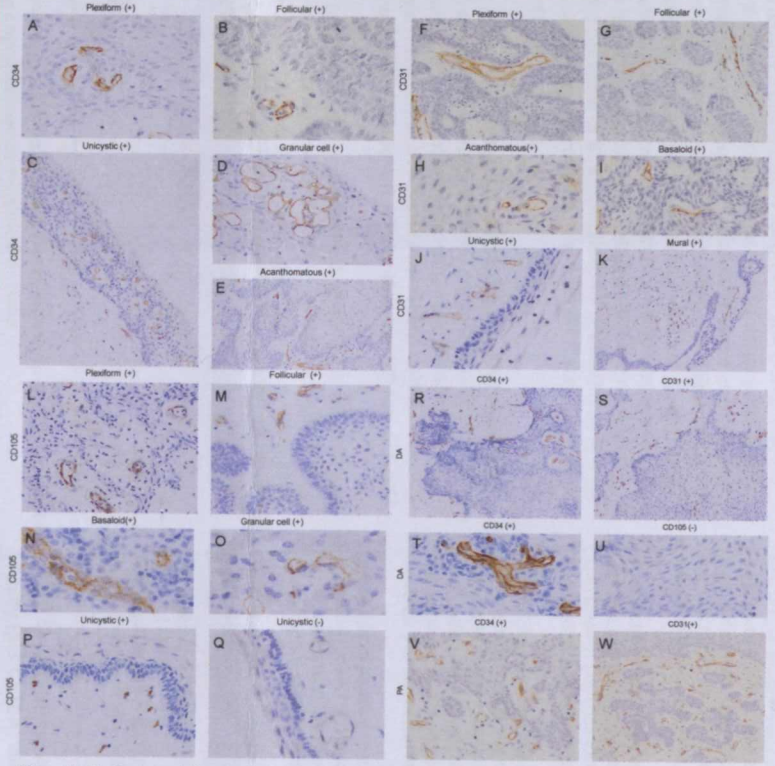
Methods:
Envision immunohistochemical technique was performed on deparaffinized 5 micron thick sections. Appropriate positive and negative controls were applied.

Expression levels were quantified using an Image Analyzer: (-) negative, (+) mild, (++) moderate and (+++) strong positivity.

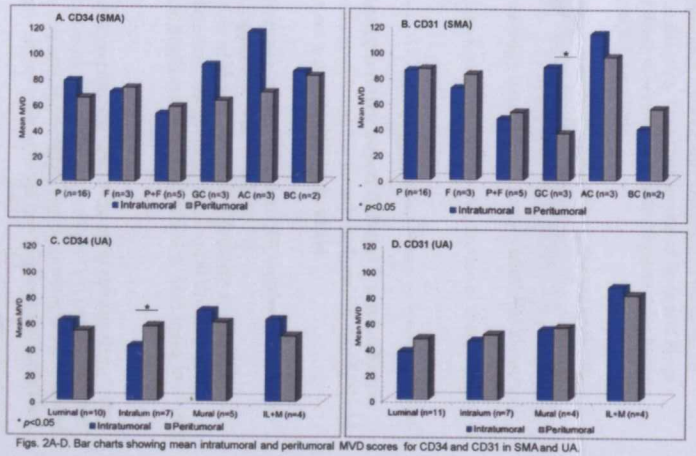
Table 2. Antibodies, clonality, catalogue ID, manufacturer and dilutions

Antibodies	*Clonality*	*Catalogue ID*	*Manufacturer*	*Dilutions*
CD34	Mouse anti-human monoclonal	M7165	Dako UK Ltd., Cambridgeshire, England, UK	1:1000
CD31	Mouse anti-human monoclonal	M0823	Dako UK Ltd., Cambridgeshire, England, UK	1:40
CD105	Mouse anti-human monoclonal	M3527	Dako UK Ltd., Cambridgeshire, England, UK	1:200

IMMUNOHISTOCHEMICAL FINDINGS



Figs. 1A-W. Representative sections of ameloblastoma subtypes showing intratumoral and peritumoral vessels stained positive for CD34, CD31 and CD105 (Original magnifications, C,E,K,R,S, x40; F,G,I,J,L,P,Q,V, W, x100; A,B,D,H,M, x200; N,O,T,U, x400).



Figs. 2A-D. Bar charts showing mean intratumoral and peritumoral MVD scores for CD34 and CD31 in SMA and UA.

Table 1. Patients' characteristics

Ameloblastoma	SMA	UA	PA	DA	RA
(n=81)	(n=32)	(n=26)	(n=4)	(n=3)	(n=16)
Mean age (Range) (yr)	31.06 (11-67)	22.1 (3-52)	43.5 (14-63)	47.7 (32-60)	40.2 (16-75)
Gender: Male/Female (n)	18/14	10/16	1/3	3/0	7/9
Race: Malays/Chinese / Indians /Others	17/13/1/1	7/15/1/3	1/0/3/0	0/1/1/1	6/8/1/1
Site: Maxilla/Mandible/NA	6/26/0	0/26	0/1/3	0/3	1/15
Xray: MLRL/ULRL/ Others /NA	8/10/6/8	6/6/7/7	0/0/0/4	2/0/0/1	2/0/1/13

SMA – Solid/Multicystic ameloblastoma, UA – Unicystic; PA –Peripheral; DA – Desmoplastic; RA – Recurrent: MLRL – Multilocular radiolucency; ULRL – Unilocular radiolucency; NA – Not available.

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3013 Intratumoral versus peritumoral mean microvessel density scores in ameloblastoma subtypes

Chong Huat Siar*, Ismadi Ishak, and Kok Han Ng
*Department of Oro-Maxillofacial Surgical and Medical Sciences, Faculty of Dentistry, University of Malaya , Malaysia

Background: Tumors require a blood supply for growth and dissemination. The ameloblastoma is a benign but locally-invasive odontogenic neoplasm with distinct behavioral characteristics of its subsets. Whether angiogenesis contributes to a more aggressive course in these variants remains unclear. The aim here was to determine and compare the intratumoral and peritumoral mean microvessel density (MVD) scores in different ameloblastoma subtypes, and to evaluate their impact on the biologic behavior of these neoplasms. Method: Study sample consisted of 81 ameloblastoma of different subtypes. Immunohistochemical staining for three vascular markers (CD31, CD34 and CD105) was performed.

Results: Solid/multicystic ameloblastoma scored a significantly higher MVD score compared to the unicystic subtype ($P<0.05$). Mean peritumoral MVD was significantly upregulated in recurrent ameloblastoma compared to primary tumors. However mean MVD scores between tumoral centre and advancing front in each ameloblastoma subtype was not significantly different ($P>0.05$).

Conclusions: Tumor angiogenesis is upregulated in ameloblastoma subtypes with a more aggressive clinical course. Lack of significant differences in mean MVD scores in the intratumoral and peritumoral locations within each ameloblastoma subtype suggest that angiogenesis-induced tumoral growth is heterogeneous in this neoplasm.

3014 Novel components of Rods and Rings – a subcellular structure with unknown function

M. Skogs[1], E.K. Lundberg[1]
[1]Science for Life Laboratory, Royal Institute of Technology (KTH), Sweden

Compartmentalization, with or without membrane enclosure, is a fundamental strategy used by cells to enhance cellular functions. Two key enzymes in the biosynthetic pathway of GTP/CTP, Inosine Monophosphate Dehydrogenase (IMPDH2) and Cytidine Triphosphate Synthetase (CTPS1), have been shown to form 2-10 µm rod- or ring-like structures in mammalian cells. The function of this structure, called Rods and Rings, is unknown but it forms in response to glutamine deprivation or by treatment with glutamine analogs and a few small molecule inhibitors.

The size of the structure, up to tenfold larger than mitochondrion, suggests that it contains multiple components. Complete knowledge of the molecular composition is an important step towards understanding its biological function. Here we describe our systematic search for additional components through the use of the Human Protein Atlas.

As part of the Human Protein Atlas database[(1)], a Subcellular Atlas[(2)] has been created with the aim to determine the subcellular location of all human genes. So far 19 000 antibodies have been analyzed by immunofluorescence in human cell lines. Antibodies giving unclassified cytoplasmic stainings with a fibrous appearance were selected for a targeted secondary screening in which 250 antibodies were re-analyzed in cell lines treated with an inhibitor that stimulates Rods and Rings formation.

We found 20 proteins that are possible novel components and three of these could be validated with two independent antibodies. Since the function of Rods and Rings is not understood the knowledge of these new components are important for further characterization and understanding of this structure.

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3015 A proteomic approach to identify host-pathogen protein-protein interactions during *Legionella pneumophila* infection

Ernest C. So, Gunnar N. Schroeder, Malgorzata Broncel, Edward W. Tate and Gad Frankel
Imperial College London, UK

Legionella pneumophila is the causative agent of Legionnaire's disease, a severe and potentially fatal pneumonia. Upon infection of alveolar cells, *Legionella* forms a unique organelle to replicate in, the *Legionella* containing vacuole. Critical to its virulence strategy is a type IV secretion system which translocates over 300 effector proteins into the host cell, inducing drastic changes to cellular signalling and the host cell proteome. Most effectors have little to no homology to known proteins and hence their function is difficult to decipher. In addition, although genetic manipulation of *Legionella* is possible, deletion of specific effectors seldom results in replication or virulence phenotypes. This is attributed to functional redundancy amongst the vast number of effectors. To shed light onto the function of these novel proteins, we have recently developed a proteomics-based method to determine the host interactomes of effectors under physiological infection conditions. Cell lines stably expressing the biotin ligase (BirA) are infected with *Legionella* strains expressing effectors fused to a Bio-tag, a tandem-affinity (TA) tag consisting of a BirA specific biotinylation site and a His_6-tag. Upon translocation into the host cell the Bio-tag is biotinylated by BirA, allowing discrimination between translocated and intrabacterial effector populations. Subsequent TA purification enables the isolation of effector-host protein complexes, whose composition are then determined by liquid chromatography tandem mass spectrometry. The effector SidM, interacts with several small Rab GTPases *in vitro* but only its interaction with Rab1 *in vivo* is well-established. Here, we used SidM to downscale our method and analyse the impact of experimental parameters such as crosslinking on the performance of the method. We determined the *in vivo* interactome of SidM which defined for the first time additional Rab GTPase targets of SidM during infection.

3016 Quantitative Mass Spectrometry based Cellular Thermal Shifts Assay (MS-CETSA) workflow development for small molecules. Analysis of the drug targets engagement on a proteome wide scale

Radoslaw Sobota[1], Yanting Lim[2] Andreas Larsson[2], Kadiam Go[2], Lekshmy Sreekumar[2], Najana Prabhu[2], Anna Jansson[2], Lingyun Dai[2], Par Nordlund [1,2,3]
[1]Systems Structural Biology, Institute of Molecular and Cell Biology, A*STAR, Singapore [2] School of Biological Sciences, Nanyang Technological University, Singapore [3]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden

Observation of the proteins thermal stability towards increasing temperature forms the foundation for methods that explore thermally induced protein unfolding. Heat treatment induced protein unfolding and aggregation can be graphically presented as a sigmoidal melting curve which allows estimation of melting temperatures (Tm).

It was shown that ligand engagement changes the functional state of the protein and induces thermal shift (Tm). This is the principle of thermal shift assays (TSA). Recent extension of this method, Cellular Thermal Shift Assay (CETSA), was developed by Nordlund's group. CETSA principle takes into account the fact that biophysical thermal stability (thermal induced unfolding) of individual proteins can be monitored and quantified in lysates/intact cells/tissue samples. TSA was previously applied on the single recombinant protein scale its extension through CETSA enabled multiple protein screening in cells using antibodies and western blot as a detection method. Further extension of the method: MS based CETSA tackles thermal shifts in whole proteome using quantitative mass spectrometry (our unpublished data and Savitski et al.,Science 2014).

Our enhanced MS-CETSA workflow, improves proteome coverage optimizing overall experimental performance and throughput. Furthermore, we developed unsupervised data analysis and visualization workflows to process and validate vast amount of MS-CETSA data. This combined strategy enabled us to monitor and validate specific drug targets in competitive and very efficient way.

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