The Hamster Model of Sequential Oral Carcinogenesis: An Update

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Abstract. *Animal models are valuable tools for studying human cancer as well as for preclinical trials. The hamster model of chemically induced sequential oral carcinogenesis was developed by our group a decade ago in order to study the multistep process of alterations in gene expression during carcinogenesis. The purpose of this review was to discuss the utility of the hamster model of sequential oral carcinogenesis regarding the deciphering of the main pathways altered. An extended search for articles that cited that specific animal models was performed. Many studies have used the hamster model of sequential oral carcinogenesis either for evaluation of the expression of biomarkers alone, or for applying chemopreventive compounds and other therapeutic methods, or combining the use of biomarkers with the anticancer effect of some compounds. It seems that this animal model is indeed a useful tool that enables the study of cell biology, pathology and therapeutics of oral cancer.*

Animal models for deciphering molecular mechanisms are widely used in biomedical research, and include insects (*Drosophila*), nematodes (*Caenorhabditis elegans*), fish (Zebrafish), and frogs (*Xenopus*), as well as many mammals, such as mice, rats, dogs, cats, pigs and monkeys (1). In

particular, the remarkable anatomical and physiological similarities between humans and other mammals, due to their phylogenetic proximity, have resulted in a better understanding of human physiology and pathology (2).

Mammalian models are particularly valuable tools for studying the cell biology and genetics of many cancer types as well as for preclinical trials of anticancer therapeutics. Two examples of animal models commonly used in cancer research are: a) Genetically modified animals, generated by a variety of interventions, such as chemical or physical mutagenesis, viral infection, transgene insertion, homologous recombination or gene editing; and b) patient-derived xenografts, where tissue or cells from a patient's tumor are implanted into an immunodeficient or 'humanized' mouse model (3). These animal models enable better study of genetic alterations and biomarkers of tumor progression since basic mechanisms of tumor formation are similar to those of human tumors. The identification of biomarkers that may serve as therapeutic targets usually requires the parallel recruitment of *in vitro* systems (primary and metastatic cell lines) in order to evaluate the efficacy of drugs before applying them to animals (3-5).

Oral cancer is a widely prevalent cancer type, encompassing about half of all head and neck cases, which constitute the sixth leading malignancy by incidence. About 90% of cases located in the oral cavity are squamous cell carcinomas (6). Despite advances in oral cancer treatment, the morbidity and mortality of oral malignancies remain high. Major risk factors for the development of oral cancer are tobacco and alcohol use, while other contributing etiological factors may include inflammation, human papillomavirus infection and genetic predisposition (6). Oral carcinogenesis is a multistep process, a result of sequential genetic alterations in oncogenes and tumor-suppressor genes,

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resulting in transformation of the oral mucosa progressively into hyperplasia, dysplasia (pre-malignant lesion clinically appearing as leukoplakia or erythroplakia), carcinoma *in situ* and invasive carcinoma (7-9).

The Hamster Model of Sequential Oral Carcinogenesis

Especially for the study of oral squamous cell carcinoma, the immunoprivileged cheek pouch of the hamster is one of the best characterized animal models (8). In particular, our group developed the hamster model of sequential oral carcinogenesis for which we obtained the first prize for basic research at an International Conference of Oral Oncology (9). Taking into consideration that molecular and cellular changes during the multistep process of oral carcinogenesis in the hamster represent changes similar to those of human oral cancer, we induced chemical carcinogenesis in the hamster in order to study different stages of tumor formation. Using the 'wiped-brush' method, we delivered the carcinogen dimethylbenz(a)anthracene (DMBA) to the left buccal pouch of each hamster and the treated buccal pouches were removed at 10, 14 and 19 weeks from the application of the carcinogen. After pathological evaluation under light microscopy, hamster tissues were classified into the following categories: Normal, hyperkeratosis, hyperplasia, dysplasia, early invasion, well-differentiated carcinoma and moderately differentiated carcinoma (Figure 1) (10).

The expression of oncogenes *Egfr*, *ErbB2*, *ErbB3*, *Fgfr2*, *Fgfr3*, *Myc*, *Nras*, *Ets1*, *Hras*, *Fos* and *Jun*, apoptosis markers Bax and Bcl2, tumor-suppressor genes *p53* and *p16* and cell proliferation marker *Ki-67* in the sequential stages of hamster oral oncogenesis was evaluated with immunohistochemical technique (9). Additional studies from our group evaluated the role of *ErbB2*, *ErbB3*, *Fgfr2*, *Fgfr3*, *c-Myc* and *p53* especially in the initial stages of oral squamous cell carcinogenesis (11-13).

Articles Citing the Foundation Report on the Hamster Model of Sequential Oral Carcinogenesis

About a decade has passed since the initial development of the hamster model of chemically induced sequential oral carcinogenesis by our group (9). Therefore, we searched for bibliographical evidence of its utility in oral cancer research by searching for the terms 'hamster' and 'sequential oral cancer'. We discuss here various references and applications of this animal model of sequential oral carcinogenesis. In addition, we briefly mention its possible use in innovative studies of gene-expression alterations in different stages of tumor formation in light of recent advancements in nanomedicine and cancer drug development.

It is well known that DMBA-induced squamous cell carcinomas in hamsters have similar morphological, histological and genetic features to human oral squamous cell carcinomas (8). Most of the recent research studies refer to our work as an example of an animal model of oral cancer (8, 13, 14-20). Some studies have used hamster models for applying therapeutic methods such as boron neutron capture therapy (14-16), photoangiolytic treatment (17), photodynamic therapy (18), or chemopreventive compounds, including resveratrol (21), medicinal herbs (22), and phenolic compounds (23) such as apigenin and carnosic acid (19) and olive oil (20).

Some research groups implemented our hamster model of sequential carcinogenesis in order to evaluate the expression of biomarkers such as P53, BCL2, RB1 and ERBB2 (24). An Indian group used the hamster model of sequential carcinogenesis in order to study the role of Wnt/ β -catenin signaling pathway during progression of oral squamous cell carcinoma. In particular, they observed abnormal accumulation of β -catenin leading to aberrant activation of the Wnt/ β -catenin signaling pathway during transformation from oral leukoplakia to dysplasia (25). Others studied the effects of down-regulation of keratin 76 in progressive oral carcinogenesis in both the hamster and mouse (26).

Several studies combined the investigation of the role of expression of certain biomarkers with the anticancer effect of some medicinal plants. For example, this hamster model of sequential carcinogenesis was used to evaluate the antitumor potential of rosmarinic acid, lyophilized strawberries, black raspberries and withaferin A in DMBA-induced hamster buccal pouch carcinogenesis based on the expression of basic genes related to tumor development (27-30). Apart from these compounds, DNA vaccines against ERBB2 and the use of virosomes encapsulated with chlorin e6 and tagged anti-EGFR antibody were applied for the purpose of improving targeting ability against oral squamous cell carcinoma (31, 32). For this purpose, they induced carcinogenesis in the hamster and classified different histological types, such as normal epithelium, low-grade (mild) dysplasia, high-grade (severe) dysplasia, and carcinoma *in situ*.

Heber *et al.* evaluated five protocols of carcinogenesis based on different number of weeks of carcinogen application to hamsters and the 6-week carcinogenesis protocol was selected for long-term studies of therapeutic effects (33). Furthermore, for the detection of oral carcinogenesis in early stages, Raman spectroscopy method was used to evaluate the levels of lipids, proteins and nucleic acids in early and late stages of oral carcinogenesis (34). Another way to detect the transformation of normal epithelium to pre-cancerous and cancerous states in early stages is non-linear optical microscopy for direct characterization of the epithelial-connective tissue interface (35). Multispectral fluorescence lifetime imaging *in vivo* and

Figure 1. Hamster model of sequential oral carcinogenesis and its utility regarding biomarkers of malignancy, metastatic capability and therapeutical targeting, based on their expression in different stages of carcinogenesis. Images obtained from (10). DMBA: Dimethylbenz(a)anthracene.

multiphoton autofluorescence micro-spectroscopy methods were used to delineate normal oral mucosa from neoplasia and differentiate between low- and high-risk oral lesions depending on the histological type (36, 37).

We and some other groups utilized our sequential carcinogenesis method in other rodents in order to evaluate the involvement of some biomarkers during cancer progression. Our group studied the influence of diabetes on signal transduction pathways in every stage of oral carcinogenesis in rats, which involved mainly an increase in cell proliferation, while no simultaneous alteration in the level of apoptosis was observed (38). Dwivedi *et al.* followed a similar approach in mice, in which they separated groups with hyperplastic and dysplastic lesions and evaluated the expression of Ki-67 and p16 (39). Similarly, de Faria *et al.* evaluated the absence of galectin-3 in the progression of tongue carcinomas in mice (40). Our group is about to conclude studies of sequential carcinogenesis on rat skin carcinoma, including basal cell carcinoma and squamous cell carcinoma (data not shown).

Future Perspectives

The ability of the sequential carcinogenesis model, through the expression of specific biomarkers, to reveal the separate stages allows new targeted therapies to be developed. One such promising method for targeted therapy is the use of nanoparticles, as described by Marcazzan *et al.* regarding inhibition of expression of specific genes (41). There are promising results using nanoplateforms for delivery of chemopreventive agents in order to reduce both *in vitro* and *in vivo* toxicity and therefore this methodology might be used to selectively inhibit relevant genes at each stage of cancer progression (41).

Based on bibliographical evidence, it seems that our rodent model of sequential carcinogenesis is indeed a very useful tool that enables the study of cell biology, pathology and therapeutics of cancer. In an era of nanomedicine and molecular pharmacology, exciting new developments are anticipated in future research and animal models of sequential carcinogenesis may possibly play a significant role in the process of deciphering the puzzle of cancer and testing therapeutical approaches.

Conflicts of Interest

The Authors have no conflicts of interest to declare in regard to this article.

Authors' Contributions

S. Kalogera searched the bibliography and produced the Figure. C. Yapijakis and S. Kalogera wrote the initial version of the article. V. Papakosta and S. Vassiliou critically corrected the article.

References

- Andersen ML and Winter LMF: Animal models in biological and biomedical research – experimental and ethical concerns. *An Acad Bras Cienc* 91: e20170238, 2017. PMID: 28876358. DOI: 10.1590/0001-3765201720170238
- Barré-Sinoussi F and Montagutelli X: Animal models are essential to biological research: issues and perspectives. *Futur Sci OA* 1: FSO63, 2015. PMID: 28031915. DOI: 10.4155/fso.15.63
- Yee NS, Ignatenko N, Finnberg N, Lee N and Stairs D: Animal models of cancer biology. *Cancer Growth Metast* 8: 115-118, 2015. PMID: 26688665. DOI: 10.4137/CGM.S37907
- Méry B, Rancoule C, Guy J-B, Espenel S, Wozny A-S, Battiston-Montagne P, Ardail D, Beuve M, Alphonse G,

- Rodriguez-Lafrasse C and Magné N: Preclinical models in HNSCC: A comprehensive review. *Oral Oncol* 65: 51-56, 2017. PMID: 28109468. DOI: 10.1016/j.oraloncology.2016.12.010
- 5 Ishida K, Tomita H, Nakashima T, Hirata A, Tanaka T, Shibata T and Hara A: Current mouse models of oral squamous cell carcinoma: Genetic and chemically induced models. *Oral Oncol* 73: 16-20, 2017. PMID: 28939069. DOI: 10.1016/j.oraloncology.2017.07.028
- 6 Tanaka T and Ishigamori R: Understanding carcinogenesis for fighting oral cancer. *J Oncol* 2011: 603740, 2011. PMID: 21772845. DOI: 10.1155/2011/603740
- 7 Farah CS, Shearston K, Nguyen AP and Kujan O: Oral carcinogenesis and malignant transformation. In: *Premalignant Conditions of the Oral Cavity*. Head and Neck Cancer Clinics. Brennan P, Aldridge T and Dwivedi R (eds.). Springer, Singapore, pp. 27-66, 2019. DOI: 10.1007/978-981-13-2931-9_3
- 8 Bähr A and Wolf E: Domestic animal models for biomedical research. *Reprod Domest Anim* 47: 59-71, 2012. DOI: 10.1111/j.1439-0531.2012.02056.x
- 9 Vairaktaris E, Spyridonidou S, Papakosta V, Vylliotis A, Lazaris A, Perrea D, Yapijakis C and Patsouris E: The hamster model of sequential oral oncogenesis. *Oral Oncol* 44: 315-324, 2008. PMID: 18061531. DOI: 10.1016/j.oraloncology.2007.08.015
- 10 Papakosta V: Immunohistochemical study of genes in hamsters and the associated mechanisms of sequential oral oncogenesis. Doctoral Thesis. National and Kapodistrian University of Athens, School of Medicine, 2007.
- 11 Vairaktaris E, Moulavassili P, Loukeri S, Spyridonidou S, Yapijakis C, Vassiliou S, Nkenke E, Vylliotis A, Papakosta V, Lazaris A and Patsouris E: Abundance and localization of skeletal muscle-related erbB2 may stimulate tumour growth during initial stages of oral oncogenesis. *J Musculoskelet Neuronal Interact* 7: 185-190. PMID: 17627089.
- 12 Vairaktaris E, Ragos V, Yapijakis C, Derka S, Vassiliou S, Nkenke E, Yannopoulos A, Spyridonidou S, Vylliotis A, Papakosta V, Loukeri S, Lazaris A, Tesseromatis C, Tsigris C and Patsouris E: FGFR-2 and -3 play an important role in initial stages of oral oncogenesis. *Anticancer Res* 26: 4217-4221. PMID: 17201136.
- 13 Papakosta V, Vairaktaris E, Vylliotis A, Derka S, Nkenke E, Vassiliou S, Lazaris A, Mourouzis C, Rallis G, Spyridonidou S, Anagnostopoulou S, Perrea D, Donta I, Yapijakis C and Patsouris E: The co-expression of c-MYC and p53 increases and reaches a plateau early in oral oncogenesis. *Anticancer Res* 26: 2957-2962. PMID: 16886620.
- 14 Monti Hughes A, Pozzi E, Thorp S, Curotto P, Medina V, Martinel Lamas D, Rivera E, Garabalino M, Farías R, Gonzalez S, Heber E, Itoiz M, Aromando R, Nigg D, Trivillin V and Schwint A: Histamine reduces boron neutron capture therapy-induced mucositis in an oral precancer model. *Oral Dis* 21: 770-777, 2015. PMID: 25926141. DOI: 10.1111/odi.12346
- 15 González SJ, Pozzi ECC, Monti Hughes A, Provenzano L, Koivunoro H, Carando DG, Thorp SI, Casal MR, Bortolussi S, Trivillin VA, Garabalino MA, Curotto P, Heber EM, Santa Cruz GA, Kankaanranta L, Joensuu H and Schwint AE: Photon iso-effective dose for cancer treatment with mixed field radiation based on dose-response assessment from human and an animal model: clinical application to boron neutron capture therapy for head and neck cancer. *Phys Med Biol* 62: 7938-7958, 2017. PMID: 28858854. DOI: 10.1088/1361-6560/aa8986
- 16 Monti Hughes A, Longhino J, Boggio E, Medina VA, Martinel Lamas DJ, Garabalino MA, Heber EM, Pozzi ECC, Itoiz ME, Aromando RF, Nigg DW, Trivillin VA and Schwint AE: Boron neutron capture therapy (BNCT) translational studies in the hamster cheek pouch model of oral cancer at the new "B2" configuration of the RA-6 nuclear reactor. *Radiat Environ Biophys* 56: 377-387, 2017. DOI: 10.1007/s00411-017-0710-9
- 17 Burns JA, Lopez-Guerra G, Kobler JB, Faquin W, LeClair M and Zeitels SM: Pulsed potassium-titanyl-phosphate laser photoangiolytic treatment of mucosal squamous cell carcinoma in the hamster cheek pouch. *Laryngoscope* 121: 942-946, 2011. PMID: 21495047. DOI: 10.1002/lary.21811
- 18 Parihar A, Dube A and Gupta PK: Photodynamic treatment of oral squamous cell carcinoma in hamster cheek pouch model using chlorin e6-histamine conjugate. *Photodiagnosis Photodyn Ther* 10: 79-86, 2013. PMID: 23465376. DOI: 10.1016/J.PDPDT.2012.05.005
- 19 Gómez-García F, López-Jornet M, Álvarez-Sánchez N, Castillo-Sánchez J, Benavente-García O and Vicente Ortega V: Effect of the phenolic compounds apigenin and carnosic acid on oral carcinogenesis in hamster induced by DMBA. *Oral Dis* 19: 279-286, 2013. PMID: 22892006. DOI: 10.1111/j.1601-0825.2012.01975.x
- 20 Soto-Castillo JJ and Ortega-Medina I: Carcinogénesis experimental con 7,12-dimetilbenzantraceno (DMBA) y su inhibición con aceite de oliva virgen extra y dieta con aceitunas maduras (variedad Picual). *Rev Española Patol* 50: 82-88, 2017. DOI: 10.1016/J.PATOL.2016.10.001
- 21 Berta GN, Salamone P, Sprio AE, Di Scipio F, Marinos LM, Sapino S, Carlotti ME, Cavalli R and Di Carlo F: Chemoprevention of 7,12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamster cheek pouch by topical application of resveratrol complexed with 2-hydroxypropyl- β -cyclodextrin. *Oral Oncol* 46: 42-48, 2010. PMID: 19932051. DOI: 10.1016/J.ORALONCOLOGY.2009.10.007
- 22 Sun Z, Guan X, Li N, Liu X and Chen X: Chemoprevention of oral cancer in animal models, and effect on leukoplakias in human patients with ZengShengPing, a mixture of medicinal herbs. *Oral Oncol* 46: 105-110, 2010. PMID: 20022553. DOI: 10.1016/J.ORALONCOLOGY.2009.06.004
- 23 Baldasquin-Caceres B, Gomez-Garcia FJ, López-Jornet P, Castillo-Sanchez J and Vicente-Ortega V: Chemopreventive potential of phenolic compounds in oral carcinogenesis. *Arch Oral Biol* 59: 1101-1107, 2014. PMID: 25033381. DOI: 10.1016/J.ARCHORALBIO.2014.06.007
- 24 Jacinto-Alemán L-F, García-Carrancá A, Leyba-Huerta E-R, Zenteno-Galindo E, Jiménez-Farfán M-D and Hernández-Guerrero J-C: ERBB expression changes in ethanol and 7,12-dimethylbenz (a)anthracene-induced oral carcinogenesis. *Med Oral Patol Oral Cir Bucal* 18: e325-331, 2013. PMID: 23229248. DOI: 10.4317/MEDORAL.18068
- 25 Vidya Priyadarsini R, Senthil Murugan R and Nagini S: Aberrant activation of Wnt/ β -catenin signaling pathway contributes to the sequential progression of DMBA-induced HBP carcinomas. *Oral Oncol* 48: 33-39, 2012. PMID: 21924667. DOI: 10.1016/j.oraloncology.2011.08.008
- 26 Ambatipudi S, Bhosale PG, Heath E, Pandey M, Kumar G, Kane S, Patil A, Maru GB, Desai RS, Watt FM and Mahimkar MB: Down-regulation of keratin 76 expression during oral carcino-

- genesis of human, hamster and mouse. *PLoS One* 8: e70688, 2013. PMID: 23936238. DOI: 10.1371/journal.pone.0070688
- 27 Anusuya C and Manoharan S: Antitumor initiating potential of rosmarinic acid in 7,12-dimethylbenz(a)anthracene-induced hamster buccal pouch carcinogenesis. *J Environ Pathol Toxicol Oncol* 30: 199-211, 2011. DOI: 10.1615/JEnvironPatholToxicolOncol.v30.i3.30
 - 28 Casto BC, Knobloch TJ, Galioto RL, Yu Z, Accurso BT and Warner BM: Chemoprevention of oral cancer by lyophilized strawberries. *Anticancer Res* 33: 4757-66, 2013. PMID: 24222110.
 - 29 Warner BM, Casto BC, Knobloch TJ, Accurso BT and Weghorst CM: Chemoprevention of oral cancer by topical application of black raspberries on high at-risk mucosa. *Oral Surg Oral Med Oral Pathol Oral Radiol* 118: 674-683, 2014. PMID: 2457886. DOI: 10.1016/j.oooo.2014.09.005
 - 30 Panjamurthy K, Manoharan S, Nirmal MR and Vellaichamy L: Protective role of Withaferin-A on immunoeexpression of p53 and bcl-2 in 7,12-dimethylbenz(a)anthracene-induced experimental oral carcinogenesis. *Invest New Drugs* 27: 447-452, 2009. PMID: 19009234. DOI: 10.1007/s10637-008-9199-z
 - 31 Berta GN, Sprio AE, Iezzi M, Spadaro M, Cappia S, Salamone P, Di Scipio F, Moggetti B, Papotti M, Musiani P, Forni G and Cavallo F: A DNA vaccine against ERBB2 impairs chemical carcinogenesis in random-bred hamsters. *Cancer Prev Res* 4: 994-1001, 2011. PMID: 21733823. DOI: 10.1158/1940-6207.CAPR-10-0301
 - 32 Low KP, Bhuvaneswari R, Thong PS, Bunte RM and Soo KC: Novel delivery of chlorin e6 using anti-EGFR antibody tagged virosomes for fluorescence diagnosis of oral cancer in a hamster cheek pouch model. *Eur J Pharm Sci* 83: 143-154, 2016. PMID: 26709081. DOI: 10.1016/j.ejps.2015.12.023
 - 33 Heber EM, Hughes AM, Pozzi ECC, Itoiz ME, Aromando RF, Molinari AJ, Garabalino MA, Nigg DW, Trivillin VA and Schwint AE: Development of a model of tissue with potentially malignant disorders (PMD) in the hamster cheek pouch to explore the long-term potential therapeutic and/or toxic effects of different therapeutic modalities. *Arch Oral Biol* 55: 46-51, 2010. PMID: 19945092. DOI: 10.1016/j.archoralbio.2009.10.010
 - 34 Kumar P, Bhattacharjee T, Ingle A, Maru G and Krishna CM: Raman spectroscopy of experimental oral carcinogenesis. *Technol Cancer Res Treat* 15: NP60-NP72, 2016. DOI: 10.1177/1533034615598622
 - 35 Pal R, Yang J, Ortiz D, Qiu S, Resto V, McCammon S and Vargas G: In-vivo nonlinear optical microscopy (NLOM) of epithelial-connective tissue interface (ECTI) reveals quantitative measures of neoplasia in hamster oral mucosa. *PLoS One* 10: e0116754, 2015. PMID: 25633927. DOI: 10.1371/journal.pone.0116754
 - 36 Malik BH, Lee J, Cheng S, Cuenca R, Jabbour JM, Cheng Y-SL, Wright JM, Ahmed B, Maitland KC and Jo JA: Objective detection of oral carcinoma with multispectral fluorescence lifetime imaging *in vivo*. *Photochem Photobiol* 92: 694-701, 2016. PMID: 27499123. DOI: 10.1111/php.12627
 - 37 Pal R, Edward K, Ma L, Qiu S and Vargas G: Spectroscopic characterization of oral epithelial dysplasia and squamous cell carcinoma using multiphoton autofluorescence microspectroscopy. *Lasers Surg Med* 49: 866-873, 2017. PMID: 28677822. DOI: 10.1002/lsm.22697
 - 38 Vairaktaris E, Spyridonidou S, Goutzanis L, Vylliotis A, Lazaris A, Donta I, Perrea D, Yapijakis C and Patsouris E: Diabetes and oral oncogenesis. *Anticancer Res* 27: 4185-4193, 2007. PMID: 18225590.
 - 39 Dwivedi PP, Mallya S and Dongari-Bagtzoglou A: A novel immunocompetent murine model for *Candida albicans*-promoted oral epithelial dysplasia. *Med Mycol* 47: 157-167, 2009. PMID: 18608888. DOI: 10.1080/13693780802165797
 - 40 de Faria PR, Chammas R, de Melo TL, Hsu DK, Liu F-T, Nonogaki S, Cardoso SV and Loyola AM: Absence of galectin-3 does not affect the development of experimental tongue carcinomas in mice. *Exp Mol Pathol* 90: 189-193, 2011. PMID: 21194530. DOI: 10.1016/j.yexmp.2010.12.007
 - 41 Marazzan S, Varoni EM, Blanco E, Lodi G and Ferrari M: Nanomedicine, an emerging therapeutic strategy for oral cancer therapy. *Oral Oncol* 76: 1-7, 2018. PMID: 29290280. DOI: 10.1016/j.oraloncology.2017.11.014

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